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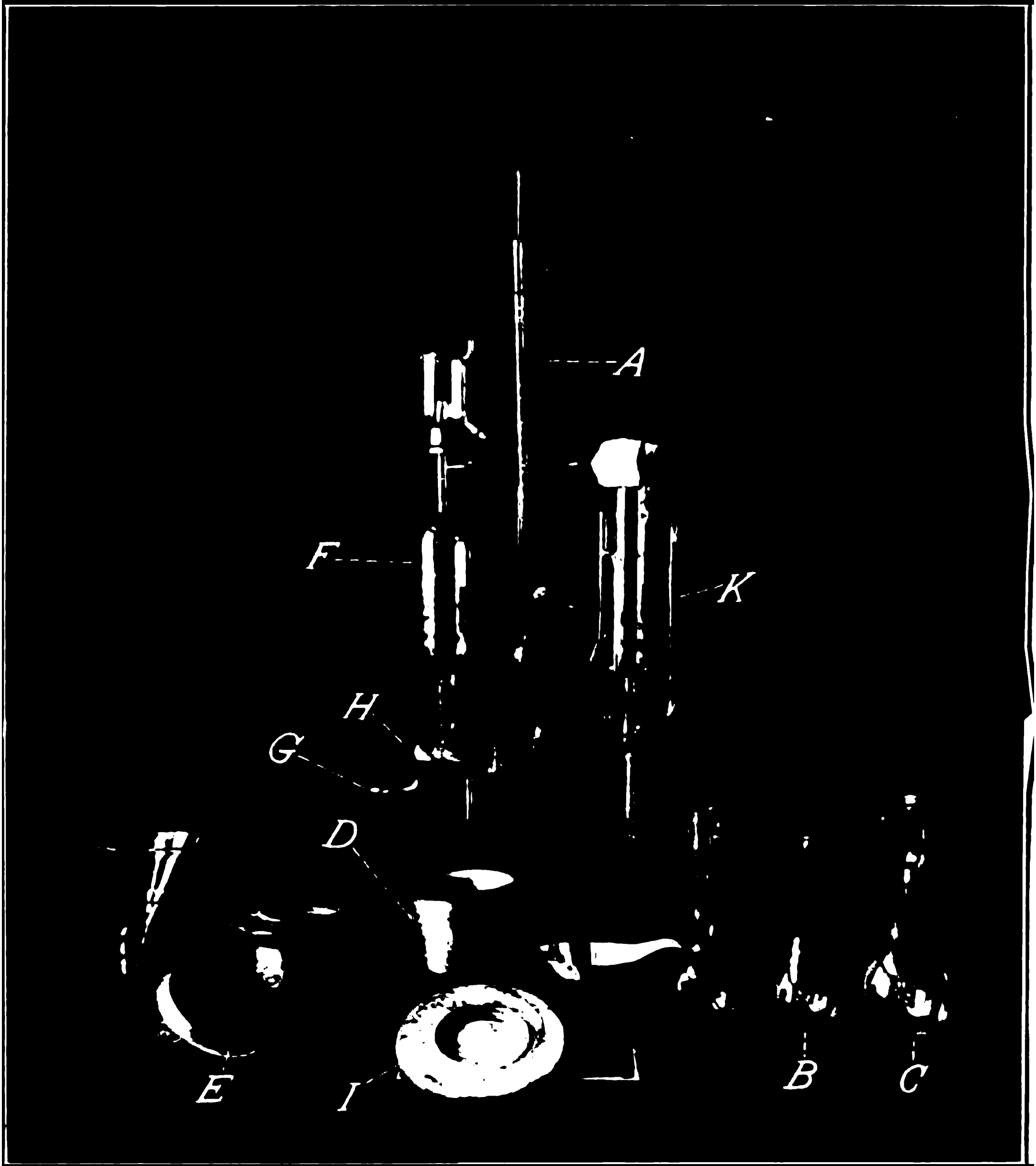

Bulletin - Bureau of Chemistry

United States. Bureau of Chemistry

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Enological Studies, No. 4. Issued November 20, 1911.

U. S. DEPARTMENT OF AGRICULTURE,

BUREAU OF CHEMISTRY—BULLETIN No. 145.

H. W. WILEY, Chief of Bureau.

ENOLOGICAL STUDIES.

THE CHEMICAL COMPOSITION OF AMERICAN GRAPES GROWN IN OHIO, NEW YORK, AND VIRGINIA.

BY

WILLIAM B. ALWOOD,
Enological Chemist.

WASHINGTON:
GOVERNMENT PRINTING OFFICE.
1911.

LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Chemistry,
Washington, D. C., March 27, 1911.

Sir: I have the honor to submit for your approval a report on the results of a three-year investigation of the composition of American grapes, conducted at Charlottesville, Va., and Sandusky, Ohio, by William B. Alwood, the enological chemist of this bureau, covering the principal commercial varieties of American grapes grown in the central and eastern sections of the country, and also other varieties not generally cultivated. These data, even though the study is far from complete, are of practical importance to the grape growers and manufacturers of grape by-products, as well as of scientific interest, and I recommend their publication as Bulletin No. 145 of the Bureau of Chemistry.

Respectfully,

H. W. Wiley,
Chief of Bureau.

Hon. James Wilson,
Secretary of Agriculture.

PREFACE.

In the conduct of certain enological work it became evident that there were almost no chemical data available regarding the character of grapes grown in Virginia, in northern Ohio, in northern and central New York, and in some other parts of the country. The condition was much the same as that which was found in the beginning of the enological studies on the production of cider. It is evident that in order to treat a subject of this kind in a rational way it is important that the character of the fruits under consideration should be definitely known. After the investigation on the composition of the grapes in the Piedmont region of Virginia was begun it was found advisable to establish a laboratory, or laboratories, for further studies on the composition of this fruit and the products made therefrom in the localities which have become so famous for the production of grapes, grape juices, and wines. The establishment of a branch of the enological investigations at Sandusky, Ohio, rendered it possible to study in situ the character of the grapes produced in that region. This study has now continued over a period of three years, and data are at hand for drawing at least preliminary conclusions respecting the general character of the fruit produced. As is the case in every grape-growing country, it has been found in northern Ohio and central and northern New York that the character of the grape varies from season to season, and often in the same vineyard or locality during a given season. This variation shows the necessity which exists in this locality, as well as everywhere else, of securing by careful selection the grapes which are to be used for specific purposes. Those that are sound, healthy, mature, and suitable for the purpose should be separated from the imperfect, unripe, damaged, or otherwise undesirable varieties. In the manufacture of unfermented grape juice, for instance, it is desirable that the grapes yield a juice which is palatable, wholesome, and easily treated. It is not desirable to bottle the fresh juices and sterilize them without removing, in so far as possible, the sedimentary matters which will be deposited on heating and standing. Many varieties of grapes lend themselves much more readily to treatment of this kind than others. The chemical composition and physical properties of the fruit and its amenability or resistance to treatment, either in the preservation of the juice in an unfermented state or in the manufacture of wine, are

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of the greatest importance. As is well known, the predominant acid in ripe grapes is tartaric. The presence of this acid, either in a free state or in combination with lime or potash, is necessary to give to the grapes the peculiar flavor for which they are noted. In other words, the acid content of the grape is one of the ingredients of chief importance from an organoleptic point of view. A grape which is without acid would be totally insipid, and would yield a juice not much better than sweetened water. On the other hand, a grape juice in which the acid is excessive is unpalatable, at least to most persons. Hence the production of a grape with the proper degree of acidity to give character and not enough to impair taste is of great importance.

The studies which have been made and which are detailed in the following pages throw much light on this important problem, even though they are far from complete, indicating what varieties of grapes and what conditions of environment give the best results.

Equally important from the point of view of vinification is the sugar content of the grape. The only source of natural alcohol in wine is fermentation of the sugars which exist in the original grape. These sugars exist in combination with other bodies, many of which enter into the finished product and serve to give it character and quality. In fact, the smaller the quantity of alcohol which can be secured in the finished wine, and at the same time permit of its manufacture and keeping, the better. Other things being equal, the wine which has the smallest percentage of alcohol is to be preferred. The data which have been secured indicate the varieties of grapes which give the best percentage of sugar for proper fermentative purposes.

These studies will lay broad and deep the foundation for the practical working out of the problem of the utilization of grapes for the various purposes for which grape products are employed. While the work has been carried on under the general direction and supervision of the Chief of the Bureau of Chemistry, the details of it and the technique of the processes have been in the hands of Mr. William B. Alwood, who has given to the work his undivided attention and has with enthusiasm and success attacked the many problems which have been presented.

H. W. WILEY,
Chief of Bureau.

WASHINGTON, D. C., *March 10, 1911.*

CONTENTS.

Page.
Object of the investigation 7
Grapes grown in Ohio and New York 8
  Methods of obtaining samples 8
  Preparation of the sample for analysis 9
  Tests and analyses made 10
  Investigation of 1908 12
  Investigation of 1909 13
  Investigation of 1910 14
  Seedlings and unknown varieties 17
Grapes grown in Virginia, 1908–1910 18
Tabulation of analytical data on samples collected at Sandusky, Ohio 20
  Data for 1908 20
  Data for 1909 21
  Data for 1910 27
Tabulation of analytical data on samples grown and analyzed at Charlottesville, Va 35

ENOLOGICAL STUDIES.

THE CHEMICAL COMPOSITION OF AMERICAN GRAPES GROWN IN OHIO, NEW YORK, AND VIRGINIA.

OBJECT OF THE INVESTIGATION.

In the discharge of duties devolving upon the Bureau of Chemistry under the food and drugs act it became apparent that definite data on the methods of wine making and of the products made in the several wine districts of the country were needed. As chemical data on the composition of the fruit used in the manufacture of grape juices and wines were not available, it was found necessary to make a fairly complete fundamental study of the grape and the by-products made from this fruit. As a first step in this direction a detailed study was made of the chemical composition and general character of the several varieties of grapes grown in the Central and Eastern States, especially those sent to the wineries at Sandusky, Ohio, with a view to determining the suitability of these grapes for food purposes and for wine production. Further than this a chemical study of the commercial wines produced in the middle and eastern wine-growing districts was also found necessary for the purpose of comparing their quality with that of wines which might be made under the direction of the bureau from the grapes commonly used at the wineries for this purpose.

The need of this investigation in the administration of the food law is readily seen if one compares the widely varying statements of the grape growers and the wine makers as to the quality of the fruit produced and the possibility of making straight wines from this fruit, and also if one is familiar with the general practice of watering and sugaring (i. e., gallizing) the wines made in the districts mentioned. For these reasons it is important to determine fully the character of the strictly nature wines made from these grapes. There appeared to be no other method of securing these data than by a thorough chemical investigation covering the whole subject of the fruit grown and the character and composition of the wines produced and sold in the districts in question. The term "American grapes" is here used to distinguish native seedlings and crosses of these with the

European species produced in America, from the distinctly European varieties grown so largely in California.

It is claimed by some that the grapes of New York State and the Atlantic seaboard States are richer in sugar and not so acid as the fruit grown in the Lake Erie district. Because of the proximity to a very large grape-growing industry and of the important wine industry located there the work has thus far been carried on chiefly at Sandusky, Ohio, but it is intended to extend it as rapidly as possible to other important centers. A large portion of the northern grape belt has already been covered in this preliminary investigation, and the importance of the crop and its by-products and the necessity for accurate information seem to fully justify this course. But the data now in hand will render possible more detailed investigations of certain specific features—as, for instance, the variability in composition of the more important varieties under differing conditions of soil, climate, and culture—which appear to promise results of much practical importance.

GRAPES GROWN IN OHIO AND NEW YORK.

METHODS OF OBTAINING SAMPLES.

In an investigation of this nature the character of the sample used is of great importance. The data for the individual samples show that the difference in composition between samples from the same source may be sufficient to be confusing. Hence, with a view to forestalling hasty conclusions, a very large number of samples, from as many sources as possible, have been examined. This should render the averages presented quite reliable. There will certainly be a very considerable variation in composition from year to year, the extent of which can only be determined by continuing these examinations until sufficient data have been secured to furnish a basis for a final statement.

The season was so far advanced when this work was begun in 1908 that arrangements could not be made to secure samples from a large number of individual growers; hence they were taken at the wine cellars. But for the years 1909 and 1910 a large number of samples were secured directly from the growers, and to check against the results so obtained samples were taken from the wine cellars. In getting samples from growers the selection of "extra quality" has been prevented as far as possible, the aim being to use the average commercial fruit. In any case the large number of samples examined from the various sources minimizes the incidental variations so as to warrant a reasonable belief that the averages herein presented represent the quality of the fruit for the season in question.

The two elements of prime importance in the grapes used for the manufacture of unfermented grape juice, for wine making, or for other by-products, are the amount of sugar and the amount of acid present in the fruit; hence the work undertaken in 1908, the first season at Sandusky, was confined almost wholly to sampling and examining crops of grapes found at the various wineries and juice factories, and to observations of the methods of vinification employed. A small amount of wine was made for technical study. It was unfortunate that the investigations could not have been begun at the opening of the vintage season in 1908, because the crop was especially good that year for making high-class products, but it was impossible to make preparations for more extensive operations than those indicated.

PREPARATION OF THE SAMPLE FOR ANALYSIS.

The samples of fruit secured for 1908 were taken at the various wine cellars directly from the packages as they arrived at the cellars, and the name and address of the grower was invariably entered on the records with each sample. About 4 pounds of fruit were taken for a sample, and care was taken to secure an average of the crop. These samples were crushed and pressed by hand in the laboratory of the Duroy & Haines Co., who kindly furnished facilities for this work.

The preparation of the samples is a matter of considerable importance. Experiments on crushing in a mortar by using a pestle, on crushing by hand in porcelain vessels, and also with small hand grinders were made. After careful tests by these methods it was found that crushing by hand is the best method of procuring a sample. In this way each grape is crushed practically to the same extent, and the whole mass is reduced to a pulp so much more homogeneous than by any other method tried, that this one was adopted for all work during the years 1909 and 1910. The stems were in every case left in the pulp.

When crushed to a pulp the sample was transferred to a piece of cheesecloth folded double, spread over a porcelain dish, and the pulp was pressed by gathering the edges of the cloth firmly in one hand and gently squeezing the mass until the juice was extracted. The pulp is in this manner reduced to a ball in the cloth, and by gripping this firmly with the free hand and applying pressure properly a careful operator can reduce the pulp to about the condition of dryness arrived at by applying 1,500 pounds direct pressure in a hydraulic press. The amount of pressure used will affect considerably the acid present in the expressed juice; hence care is required in this manipulation to secure comparable results.

TESTS AND ANALYSES MADE.

Each sample of juice was then tested as to density with a Brix spindle, and in most instances a sample was saved for further examination. These samples for the year 1908 were bottled and preserved with mercuric chlorid, using tablets purchased at the drug store, and in this condition they were sent to Stonehenge laboratory, Charlottesville, Va., for analysis. The final analyses were completed by January 5, 1909, and covered only specific gravity, total solids, sugar-free solids, total sugar, and fixed, volatile, and total acid. These data were carefully checked, and in the acid determinations allowance was made for the use of the mercuric chlorid tablets, each of which contained 0.5 gram of mercuric chlorid and 0.57 gram of ammonium chlorid. One tablet was used for 400 cc of fresh juice. A correction to allow for the acid present in the tablet was made by deducting 0.075 gram tartaric acid per 100 cc of sample. Volatile acid was determined on the samples taken in 1908, but the amount was so slight that it is not reported in the tables; seldom was 0.01 gram per 100 cc of sample found. During the years 1909 and 1910 volatile acid determinations were not made.

The results on total acid for the samples taken in 1908 are surprisingly low. This was noted as the samples were analyzed, and the work was carefully checked. The only explanation which is suggested is that during the first season the samples were not pressed sufficiently to bring out all the acid present. It will be readily understood that for red wines the acid content secured by crushing and pressing the fresh fruit will not be the total acid secured by the fermentation of the pulp and then pressing by power, as is customary in the wineries, but for white wine the result should be practically the same as when the fruit is crushed and pressed by power. These points will be covered and the data given in an additional report on the fermentation experiments conducted at the Sandusky laboratory.

The preparation of the samples during the years 1909 and 1910 was uniformly by hand as just described; but, having equipped a field laboratory at Sandusky, the analyses of the juice were made there at once, as far as possible. In every case the acid was determined by the titration of the freshly pressed and filtered juice, and a gravity reading was made on unfiltered juice at the same time, using a Brix spindle specially made for this work. When the analysis could not be made promptly a sample was preserved in a pint champagne bottle with 0.2 gram of mercuric chlorid. The full analysis was completed usually within from 30 to 60 days after sampling. If the presence of sucrose was suspected the analysis was made at once, because experience showed that inversion of this form of sugar occurred even in the chemically preserved sample.

The methods of analysis followed are those laid down in Bulletin No. 107, Revised, Bureau of Chemistry; certain details for which no explicit directions are given were made to conform to well settled laboratory practice. Total solids were derived as for wine from the table given in Bulletin No. 107, Revised (p. 218 et seq.), and the sugar-free solids by subtracting the total reducing sugar from the total solids, save in cases where the sample showed sucrose. In such instances the sugar-free solids were obtained by subtracting the sum of the reducing sugar and sucrose from the total solids. Subtracting the total sugar found by inversion when sucrose is present results in reducing the sugar-free solids below normal.

The acid determination was made on filtered juice without previous heating, by titrating with tenth-normal sodium hydroxid, free from carbonate, using a neutral solution of azolitmin on a spot plate as indicator. When the juice was highly colored it was found advantageous to dilute the portion taken about two and one-half times with recently boiled but cold water, that the reaction might be more clearly seen.

The sugar determinations were made after the methods of Munson and Walker (Bulletin No. 107, Revised, p. 241), but it was found possible to use the gooches twice without cleaning. This was definitely determined by trial, as shown by the following check results:

Reducing sugar determinations to test need of cleansing gooch before each determination.

[Grams per 100 cc.]

Filtered and weighed on clean pad:		Filtered and weighed on 4.3 decigrams cuprous oxid:	
White grape juice—		White grape juice—	
1	21.11	5	21.01
2	21.13	6	21.05
3	21.11	7	21.08
4	21.11	8	21.08
Average	21.12	Average	21.06
Red grape juice—		Red grape juice—	
1	16.88	5	16.83
2	16.87	6	16.91
3	16.85	7	16.85
4	16.91	8	16.83
Average	16.88	Average	16.86

The Brix readings given are those made on the fresh juice, with a standardized Brix must spindle especially devised by this laboratory and corrected to 20° C. in accordance with the regulations of the Bureau of Standards. The specific gravity readings given were in every case determined by pyknometer when the sample was analyzed. These notes apply to the work of 1909 and 1910; some slight variations from this detail occurred during the preliminary work of 1908.

INVESTIGATION OF 1908.

The data for each variety sampled in 1908 are summarized in Table I, page 20, by bringing the results on all the samples of a variety from each locality together and giving the average; if more than one sample was analyzed, the maximum and minimum results for each locality are shown. The figures presented in this table cover too few samples to warrant any extended discussion; from several important localities only one sample of a given variety was examined. This was due to lack of time to organize the work completely for that year. Further, the data are deficient in that the territory was not adequately covered. These criticisms, however, do not deprive these data of value, and, as far as they go, the results show a remarkably high sugar content in the great majority of the samples analyzed. In fact, the grape crop was of very good quality that year, and it was often stated that not in 20 years could such quality be expected again. Only one sample of Catawba was analyzed from Middle Bass and one from North Bass, Ohio, but the Brix readings on the other samples from these localities show that they were all richer in solid matter and hence presumably were higher in sugar than the two analyzed. The importance of the Brix readings of fresh must, as a means by which the grower can determine with considerable accuracy the sugar content of the fruit, is worthy of special attention.

The data[1] show that 19 samples of Ives, notably the poorest grape in sugar content of those used for wine in the Sandusky district, gave an average percentage composition of 15.06 per cent of sugar and 0.485 of total acid. Concord, which is usually not much superior to Ives, shows for 11 samples 16.20 per cent of sugar and 0.434 of total acid. Clinton, a variety not largely grown, showed in 5 samples 17.76 per cent of sugar and 0.998 of total acid. Norton, a grape apparently not well adapted to the Lake district, gave for 3 samples an average of 22.79 per cent of sugar and 0.907 of total acid. The fermented pulp will, of course, give a wine that is still more acid.

It is especially important, however, to note that Catawba, the chief white wine grape of the Lake district, on an average of 25 samples, showed 18.81 per cent of sugar and 0.750 of total acid. Delaware, which is well known for its high quality, showed for 2 samples an average of 22.84 per cent of sugar and 0.423 of total acid; and Iona, a superb wine grape, but not extensively grown, in one sample had 19.35 per cent of sugar and 0.623 of total acid. These results as to sugar content indicate a very high quality of fruit.

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[1] For percentage data see table on page 16.

INVESTIGATION OF 1909.

During the season of 1909 preparation was made to extend the work by equipping a laboratory at Sandusky, Ohio. Accommodation was thus provided for handling a large number of samples and for making fermentation experiments on the different varieties of wine grapes. The report on the fermentation experiments is not, however, included in this report, which deals only with the composition of the fruit samples. A canvass of the Lake Erie district and as far east as the central New York grape district was made during August and September, 1909, and correspondents were selected at several points of importance who were relied upon for samples of the crop for the laboratory work, and a number of whom rendered valuable assistance. The samples were sent by express or boat, usually in small baskets, to the Sandusky laboratory, where the notes on quality and the chemical examinations were made.

This arrangement furnished a large number of samples direct from the growers, but others were also secured from the wine cellars in Sandusky at such periods as seemed most likely to furnish data on the full range of the fruit crushed for wine. However, samples from the wine cellars were not usually considered as representing individual crops during 1909, but were regarded as a composite of the stock on hand at the time the sample was taken. From the American Wine Company composite samples were taken of the expressed juice as it was collected in the settling tank. Such samples are reported under the names of the several cellars.

The data determined at the time of sampling during 1909 comprise a Brix reading on the fresh must, and titration for total acid as soon as the sample was crushed and pressed; in most cases the sample was further analyzed by determining the specific gravity by pyknometer, the total solids, sugar-free solids, and total sugar. Inversion was only resorted to when the results showed excessive sugar-free solids by direct determination. Volatile acid was not determined on the fresh fruit during 1909, as the data for the previous year showed this to be unimportant. Samples which were not analyzed as fresh fruit were treated with pure mercuric chlorid and stored for analysis later, 0.2 gram being used for 400 cc of juice. A correction for the use of mercuric chlorid was found to be unnecessary when the pure crystallized salt is used. The results on acid in a juice are usually lower if the sample has stood in a bottle for some time, owing to the precipitation of crude "tartar." Tests on a number of varieties showed this to amount approximately to 0.064 gram per 100 cc for white juice and 0.051 gram for red juice. The results given in the table are not, however, affected by this fact, because the acid was determined in the fresh juice.

The results for 1909 are summarized by varieties, bringing together all the samples collected in each locality. These data are found in Table II (p. 21). The composite samples from the individual wine cellars are arranged together without reference to the locality from which the crops came.

The year 1909 was remarkable for the very heavy crop of fruit borne on the vines. It was commonly remarked by the growers that such an abundant crop had seldom been produced. But unfortunately the latter part of the season was very unpropitious for the ripening of the load of fruit with which the Catawba vines were weighed down. Cold, rainy weather set in when the main crop was barely colored, and this continued with slight intermission until actual freezes injured the foliage, with the result that in some vineyards a considerable portion of the crop did not mature and was either a total loss or was sold at low prices—from $12 to $15 per ton. A considerable number of these pale Catawba samples were tested for sugar and acid and found to run from 2 to 4 per cent lower in sugar and about 2 per mille, i. e., per 1,000, or 0.2 per cent, higher in acid than the average mature grapes. In 1909 every variety to which special attention has been called (namely, Clinton, Concord, Ives, and Norton of the red-juice grapes, and Catawba, Delaware, and Iona of the white-juice grapes), shows a sugar content from 1 to 4 per cent lower than in 1908, and the acid is higher. Yet even in this admittedly very unfavorable year the grapes were, on the whole, of a good quality. It is notable that out of 16 samples of Catawba juice secured from a single cellar only 2 fell below the average of 16.31 per cent of sugar for the entire 84 samples from all sources in Ohio, and that the acid was only slightly higher than the average in 8 instances out of the 16. Thus even in a bad crop year it is shown that fruit of very fair quality can be selected. The 16 juice samples mentioned were from selected crops bought for making champagne wine and indicate that the quality of the fruit can be greatly affected by the method of growing the crop.

INVESTIGATION OF 1910.

The season of 1910 was marked by the almost total failure of the grape crop in some sections of the northern fruit belt, due to the severe late spring frosts. However, the Lake Erie and central New York districts gave about 40 to 60 per cent of a crop. On the whole the quality of the crop was in most instances quite equal to that of 1908. The sampling work was extended to cover more fully the two districts mentioned, and a few samples of Concord grapes were secured from Michigan. The manipulation of the samples was the same as previously noted, and all deferred analyses were completed by December 20, 1910. A large number of varieties were analyzed which, judging from the results, are of little importance for the manufacture of grape products, but it was thought best to include in the investigation all varieties found in the districts covered for a period of several years, so that the data accumulated might be exhaustive.

During the season of 1910 the work was furthered by the hearty cooperation of the New York Agricultural Experiment Station, both at the main station at Geneva and at the grape station at Fredonia, N. Y. Prof. Hedrick and his colleagues collected and forwarded all the samples from Geneva and from the Chautauqua grape belt. In the Lake Keuka district the very efficient cooperation of Mr. L. D. Masson at Hammondsport, N. Y., and of Mr. W. N. Wise at Penn Yan, N. Y., was secured. For the important grape district about North East, Pa., Mr. Fred Johnson, agent and expert for the Bureau of Entomology, in charge of the investigation of grape insects at that point, was authorized to collect and forward samples of fruit to the Sandusky laboratory, which he did in a most satisfactory manner. For the Lake Erie islands Mr. Henry Elfers at Kelleys Island, Mr. H. J. Burns at Middle Bass, and Mr. R. Siefield at North Bass, rendered very valuable assistance in collecting and forwarding samples.

The tabular presentation of the analyses for 1910 follows the same plan as that of the two previous years and is sufficiently comprehensive to warrant careful study both by the grape grower and the manufacturer of grape by-products. (See Table III, p. 27.) It is not warrantable at this time to theorize on the results of these three years of investigation further than to point out the remarkably good quality of such varieties as Catawba, Delaware, Iona, and others of the grapes with light-colored juice and also the rather unexpected high sugar content shown by Concord. Norton and Clinton have a very good percentage of sugar, but such a very high percentage of acid as to render their value for the northern grape belt problematical. But Montefiore, which is so little grown that it could not be studied sufficiently to determine its value with any certainty, has much promise because of the low acid and the high sugar content. If it can be successfully cultivated this should prove an important grape.

A tabular statement of the average sugar and acid content for four of the red and three of the white juice grapes commercially accepted as the best for wine making in the northern grape belt is given in the following table, which covers the data for the three years during which the work has been in progress. During the first year only Ohio samples were examined, but after that time samples from other localities were included, except in the case of Norton. The Ohio samples are given separately and then the data for all of the samples together, since that district is so much better represented in most instances, and also, thus far, presents some indications of a higher sugar and lower acid content than fruit from the other States. This is an interesting point, but it must be confirmed by a more complete examination of fruit from the other sources before a definite statement can be made.

The varieties compared include, as stated, some of the most important commercial varieties, and also represent the three groups of native grapes from which our cultivated varieties are mostly derived, namely, the Riparia, Aestivalis, and Labrusca groups, as well as Vinifera hybrids. Further investigations, dealing more critically with a large number of varieties of each of these groups, are much needed.

The Delaware and Iona (Labrusca and Vinifera hybrids) have a remarkably high sugar and low acid content; Catawba, a Labrusca, with possibly a strain of Vinifera parentage, shows fairly high sugar and also high acid; the Ives and Concord, pure natives of the Labrusca group, are low in acid and not sufficiently high in sugar to make a claret wine of the usual strength; that is, from 9 to 10 per cent of alcohol. Clinton represents the Riparia and Norton the Aestivalis group of our native species. Both of these can be grown with sufficient sugar content to make a fine, sound wine, but they are strongly acid.

Comparison of average sugar and acid content of important varieties for three years.

[Percentage data calculated from results expressed in grams per 100 cc (Tables I-III) by dividing by the specific gravity.]

Variety and year.	Samples from Ohio.				Samples from all sources.			
	Total sugar.		Total acid.		Total sugar.		Total acid.	
	Number of samples.	Amount.	Number of samples.	Amount.	Number of samples.	Amount.	Number of samples.	Amount.
Clinton:		*Per cent.*		*Per cent.*		*Per cent.*		*Per cent.*
1908	5	17.76	5	0.998	...	...	...	...
1909	12	16.14	12	1.553	16	16.39	16	1.638
1910	3	18.99	3	1.755	7	18.12	7	1.872
Concord:								
1908	11	16.20	11	.434	...	...	...	...
1909	46	14.92	40	.646	76	14.89	69	.681
1910	30	16.77	30	.693	88	15.75	88	.785
Ives:								
1908	19	15.06	19	.485	...	...	...	...
1909	27	14.05	26	.721	30	13.76	29	.720
1910	18	14.79	18	.693	22	14.82	22	.720
Norton:								
1908	3	22.79	3	.907	...	...	...	...
1909	11	17.84	11	1.657	...	...	...	...
1910	5	19.53	5	1.534	...	...	...	...
Catawba:								
1908	25	18.81	25	.750	...	...	...	...
1909	84	16.31	84	1.067	91	16.59	91	1.080
1910	84	19.20	84	.984	93	19.01	93	1.016
Delaware:								
1908	2	22.84	2	.423	...	...	...	...
1909	22	18.96	17	.748	36	18.92	31	.762
1910	11	22.02	11	.720	28	19.57	28	.803
Iona:								
1908	1	19.35	1	.623	...	...	...	...
1909	3	17.82	3	.757	4	18.18	4	.770
1910	5	19.51	5	.793	8	19.16	8	.921

SEEDLINGS AND UNKNOWN VARIETIES.

In covering as large a territory as that from which the samples listed in this report have been collected, naturally a number of seedlings or unknown varieties have been received. Of these, nine appear to merit special attention. For convenience only, these are numbered from 1 to 9.

The first mentioned in the table which follows was found in the vineyard of Mr. N. Schneider at Vermilion, Ohio. He states that it is a seedling grown by him some years ago direct from Catawba, and the characteristics of the vine and the fruit bear out his statement as to parentage. It has a more neutral flavor than Catawba and when fully ripe tastes less acid, but the analyses do not show much in its favor as compared with the parent stock. However, its vinous character deserves attention.

The second of these seedlings was found in the vineyard of Mr. E. L. Steuk, at Venice, Ohio, and is of unknown parentage. The full discussion of this grape given in Bureau of Chemistry Bulletin 140 renders further consideration unnecessary. Yet it is important to call attention to its very low acid and fairly high sugar content. The flavor is very mild, almost insipid when ripe. The fruit is somewhat of the Iona type.

The seedling secured from Mr. J. H. Bauman, Kelleys Island, Ohio, is high in sugar and has a very desirable type of bunch, the fruit is red in color, with white juice and good flavor. No sample of this fruit was secured in 1910.

The sample from Mr. L. C. Dodge, Middle Bass Island, Ohio, did not show up well in the analysis for 1909, but in 1910 it had a high sugar content, low acid, and mild flavor. The fruit is like Catawba in type, and it is perhaps a seedling of this variety.

The seedling from Mr. N. Wagner, Brownhelm, Ohio, showed remarkably high sugar and low acid for the year 1909. No sample was secured in 1910.

The sample from Mr. H. Kümmel, Middle Bass Island, Ohio, shows only a fair amount of sugar, but the acid is low and the flavor very good, being mild and vinous. The fruit is black, and the bunches compact, of the Norton type. Such a grape might be desirable to mix with Clinton or Norton.

The seedling secured from Mr. H. Beatty, Kelleys Island, Ohio, showed very high sugar for 1910, and low acid in both years. It has loose bunches, black fruit with white juice, is mild in flavor, and might be desirable for blending. Its parentage is unknown.

From Mr. J. Schonhardt, Venice, Ohio, two samples were obtained, numbered 8 and 9 in the table. No. 8 is like Concord in type of bunch and in color, but is very much higher in sugar content, with a moderately low acid. No. 9 is Catawba in type, with a remarkably high sugar and low acid. If these last have such growth characteristics as to render their culture practicable they can not fail to be of commercial value.

Sugar and acid content of seedlings or unknown varieties.

Seedling No.	Field No.	Grower and address.	Date of sampling.	Total sugar as invert.		Acid as tartaric.	
				Grams.	Per cent.	Grams.	Per cent.
1	195	N. Schneider, Vermilion, Ohio	Oct. 7, 1909	21.21	19.42	0.979	0.897
1	91	.....do	Sept. 24, 1910	20.00	18.40	1.144	1.053
1	204	.....do	Oct. 2, 1910	19.95	18.35	1.005	.925
2	122	E. L. Steuk, Venice, Ohio	Oct. 1, 1909	19.52	17.99	.521	.480
2	179	.....do	Oct. 6, 1909	17.41	16.21	.473	.440
2	241	.....do	Oct. 9, 1909	18.78	18.43	.450	.416
2	50	.....do	Sept. 16, 1910	19.96	17.42	.529	.418
2	131	.....do	Sept. 26, 1910	22.37	20.45	.439	.401
3	361	J. H. Bauman, Kelleys Island, Ohio	Oct. 20, 1909	20.43	18.76	.938	.861
4	491	L. C. Dodge, Middle Bass, Ohio	Oct. 27, 1909	13.44	12.64	1.301	1.223
4	53	.....do	Sept. 17, 1910	19.91	18.32	.743	.684
5	331	N. Wagner, Brownhelm, Ohio	Oct. 17, 1909	19.08	17.56	.754	.694
6	203	H. Kümmel, Middle Bass, Ohio	Oct. 7, 1909	18.37	16.90	.514	.473
6	69	.....do	Sept. 21, 1910	17.50	16.25	.664	.616
7	71	H. Beatty, Kelleys Island, Ohio	Sept. 24, 1909	18.41	17.05	.656	.607
7	348	.....do	Oct. 8, 1910	24.39	22.13	.626	.568
8	41	J. Schonhardt, Venice, Ohio	Sept. 16, 1910	22.43	20.45	.848	.763
9	229	.....do	Oct. 3, 1910	26.11	23.54	.683	.616

GRAPES GROWN IN VIRGINIA, 1908–1910.

Formerly Charlottesville, Va., was a grape-growing center of considerable importance, but the very destructive ravages of black rot from 1886 to 1890, before the present methods of controlling this disease were developed, among other factors resulted in greatly reducing the area of grapes in cultivation. There remains, however, a small but important industry which is confined chiefly to growing Norton grapes for wine in the Piedmont section of Virginia. This district is the natural home of the Norton, where, under careful cultivation, it grows to a perfection seldom reached elsewhere. With the exception of this variety, the grape-growing industry is of very little importance in the Virginia Piedmont. During the years 1909 and 1910, however, samples were obtained of all of the other varieties obtainable from the local wine cellars at Charlottesville.

The Norton is, when matured properly, such a valuable grape for red wine and for grape-juice manufacture that it is worthy of a thorough investigation. There is also every reason to believe that most of the commonly grown American grapes can be produced in the Virginia Piedmont of a quality quite equal to those of other sections, but the results show that at present this is not done. However, in the case of the Norton the high content of sugar and the low acid indicate a grape superior, in the main, to the samples of this variety from other districts.

The climate and soil conditions of the Virginia Piedmont are so different from those of the northern grape belt that it seems best to present the results for this district in a separate table. It is intended to extend the investigation of the composition of grapes grown in the Atlantic Coast States so as to cover the territory more thoroughly and to secure a larger number of samples. The data here presented are not sufficient to warrant conclusions, but they are interesting and show the possibility of producing a very high quality in at least some of the black grapes grown in this section.

The samples analyzed at the Stonehenge Laboratory were obtained through the courtesy of Mr. A. Russow, of the Monticello Wine Co., and were taken by him from the fruit brought to the wine cellar at vintage time. It was endeavored to make the sample represent the crop as delivered.

For 1908 only samples of Norton were obtained. These show a very high average sugar content and an average acid content of less than 1 per cent. This is certainly a high quality and warrants the statement that such a juice needs very little change in composition for wine making. The maximum sugar content found of 22.34 per cent, with 0.902 of total acid, is phenomenal for a strictly American grape. A comparison of the three years' results on Norton shows that the same vineyard has in each year yielded the maximum quality, the average for the three years being above 22 per cent of sugar and 8 per mille of acid (i. e., per thousand, or 0.8 per cent). In several instances the sampling of the Norton grapes covers a period of from four to seven days, yet there is no marked difference shown in composition. The question of the changes in percentage of acid and sugar in the grapes grown in this district needs a thorough investigation.

During 1909 and 1910 the sampling of several crops of Concord grapes also covered a period from four to seven days, and decided improvement was shown in some of these crops during this time, while in other cases it was slight. The sampling of several crops of Ives for like periods shows no decided results in favor of the later pickings, and in some cases a deterioration is indicated. These data are by no means to be accepted as proof that the crops actually deteriorated. The question needs a careful investigation of reserved vines, which can be studied for a much longer period, before it can be definitely answered. In the opinion of the writer none of the results for Concord and Ives show the quality which may be produced by thoroughly maturing these varieties. Table IV (p. 35) presents the average data obtained on these Virginia grapes.

TABULATION OF ANALYTICAL DATA ON SAMPLES COLLECTED AT SANDUSKY, OHIO.

DATA FOR 1908.

TABLE I.—*Average, maximum, and minimum data for 1908, arranged by varieties and localities. (Analyses by Hartmann and Eoff.)*

[Results expressed in grams per 100 cc of expressed juice.]

Variety and locality.	Number of samples.	Number of growers.	Brix.	Total solids.			Sugar-free solids.			Sugar as invert.			Acid as tartaric.		
				Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.
BACCHUS.															
Vermilion, Ohio	2	1	22.1	23.28	23.46	23.09	2.77	2.82	2.72	20.51	20.74	20.27	1.205	1.215	1.196
CATAWBA.															
Kelleys Island, Ohio	14	10	21.2	22.98	24.67	21.83	2.21	2.39	1.94	20.77	22.73	19.48	.825	1.013	.675
Middle Bass, Ohio	1	1	19.9	21.70	.....	.....	2.13	.....	.....	19.57	.....	.....	.743	.....	.....
North Bass, Ohio	1	1	19.3	20.89	.....	.....	2.09	.....	.....	18.80	.....	.....	.855	.....	.....
Port Clinton, Ohio	2	2	21.6	22.42	23.25	21.59	2.11	2.23	2.00	20.30	21.02	19.59	.769	.788	.750
Put in Bay, Ohio	6	6	21.1	22.74	24.57	21.59	2.23	2.70	1.87	20.51	22.70	19.57	.839	.971	.668
Vermilion, Ohio	1	1	19.1	20.41	.....	.....	2.00	.....	.....	18.41	.....	.....	.638	.....	.....
Averages, limits, and totals	25	21	20.9	22.64	24.67	21.59	2.19	2.70	1.87	20.45	22.73	19.48	.775	1.013	.668
CLINTON.															
Huron, Ohio	3	1	22.5	23.45	24.04	22.54	2.64	2.81	2.49	20.81	21.43	19.73	.975	1.125	.863
North Bass, Ohio	1	1	18.9	19.21	.....	.....	2.78	.....	.....	16.43	.....	.....	1.265	.....	.....
Vermilion, Ohio	1	1	19.7	20.07	.....	.....	2.56	.....	.....	17.51	.....	.....	1.215	.....	.....
Averages, limits, and totals	5	3	21.2	21.93	24.04	22.54	2.65	2.81	2.49	19.28	21.43	19.73	1.081	1.125	.865
CONCORD.															
Berlin Heights, Ohio	1	1	19.8	20.73	.....	.....	1.85	.....	.....	18.80	.....	.....	.473	.....	.....
Brownhelm, Ohio	1	1	18.2	18.74	.....	.....	1.68	.....	.....	17.06	.....	.....	.488	.....	.....
Kelleys Island, Ohio	2	2	19.5	20.57	20.83	20.31	1.90	2.29	1.51	18.67	19.32	18.02	.426	.473	.379
Marblehead, Ohio	1	1	17.2	18.16	.....	.....	1.21	.....	.....	16.95	.....	.....	.349	.....	.....
North Bass, Ohio	2	2	18.6	19.89	20.07	19.52	1.69	1.79	1.59	18.10	18.48	17.73	.488	.578	.398
Sandusky, Ohio	1	1	17.6	17.83	.....	.....	2.08	.....	.....	15.75	.....	.....	.454	.....	.....
Vermilion, Ohio	2	2	17.6	18.74	20.60	16.88	1.74	2.03	1.46	16.99	18.57	15.42	.495	.521	.469
Locality not given	1	1	16.7	16.70	.....	.....	1.40	.....	.....	15.30	.....	.....	.544	.....	.....
Averages, limits, and totals	11	11	18.3	19.12	20.83	16.88	1.72	2.29	1.46	17.40	19.32	15.42	.466	.578	.379
DELAWARE.															
North Bass, Ohio	2	1	24.8	27.27	28.46	26.09	2.03	2.28	1.78	25.24	26.18	24.31	.467	.495	.439
IONA.															
Vermilion, Ohio	1	1	21.6	23.30	.....	.....	2.21	.....	.....	21.09	.....	.....	.679	.....	.....
ISABELLA.															
Kelleys Island, Ohio	2	2	20.3	21.83	21.86	21.80	1.95	2.03	1.86	19.89	19.94	19.83	.540	.656	.424
IVES.															
Catawba Island, Ohio	1	1	16.6	17.64	.....	.....	1.80	.....	.....	15.84	.....	.....	.379	.....	.....
Ceylon, Ohio	4	3	17.0	18.37	18.66	16.13	1.87	1.98	1.75	15.49	16.91	14.28	.553	.608	.461
Danbury, Ohio	2	2	17.4	18.28	19.00	17.56	2.05	2.06	2.04	16.23	16.96	15.50	.506	.525	.488
Huron, Ohio	3	2	19.1	21.19	22.72	18.48	2.09	2.46	1.50	19.10	21.22	16.16	.490	.555	.375
Marblehead, Ohio	1	1	16.8	17.48	.....	.....	1.88	.....	.....	15.60	.....	.....	.514	.....	.....
Put in Bay, Ohio	2	1	17.2	18.03	18.14	17.93	2.09	2.40	1.78	15.94	16.36	15.53	.603	.776	.431
Vermilion, Ohio	6	6	16.7	17.16	19.68	15.71	1.96	2.11	1.79	15.19	17.74	13.62	.510	.551	.431
Averages, limits, and totals	19	16	17.9	18.09	22.72	15.71	1.97	2.46	1.50	16.12	21.22	13.62	.519	.776	.375

TABLE I.—*Average, maximum, and minimum data for 1908, arranged by varieties and localities*—Continued.

Variety and locality.	Number of samples.	Number of growers.	Brix.	Total solids.			Sugar-free solids.			Sugar as invert.			Acid as tartaric.		
				Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.
MONTEFIORE.															
Kelleys Island, Ohio	1	1	(1)	26.62	.....	.....	1.82	.....	.....	24.80	.....	.....	.401	.....	.....
NORTON.															
Huron, Ohio	1	1	25.4	27.17	.....	.....	3.06	.....	.....	24.12	.....	.....	1.013	.....	.....
Kelleys Island, Ohio	1	1	28.6	30.55	.....	.....	3.23	.....	.....	27.32	.....	.....	1.010	.....	.....
Venice, Ohio	1	1	25.4	27.38	.....	.....	3.00	.....	.....	24.38	.....	.....	.994	.....	.....
Averages, limits, and totals	3	3	26.5	28.37	.....	.....	3.09	.....	.....	25.27	.....	.....	1.006	.....	.....
RIESLING.															
Kelleys Island, Ohio	1	1	.....	20.65	.....	.....	2.08	.....	.....	18.57	.....	.....	.656	.....	.....
SCHRAIDT.															
North Bass, Ohio	1	1	.....	21.20	.....	.....	2.97	.....	.....	18.23	.....	.....	1.294	.....	.....
SEEDLING (SCHNEIDER)															
Vermilion, Ohio	1	1	25.9	28.17	.....	.....	2.33	.....	.....	25.84	.....	.....	.540	.....	.....

[1] Brix not read.

DATA FOR 1909.

TABLE II.—*Average, maximum, and minimum data for 1909, arranged by varieties and localities. (Analyses by Hartmann and Eoff.)*

[Results expressed in grams per 100 cc of expressed juice.]

Variety and locality.	Number of samples.	Number of growers.	Average Brix.	Total solids.			Sugar-free solids.			Sugar as invert.			Acid as tartaric		
				Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.
AGAWAM.															
North East, Pa	1	1	16.7	17.60	.....	.....	2.15	.....	.....	15.54	.....	.....	1.043	.....	.....
Ripley, N. Y	1	1	(1)	22.07	.....	.....	1.81	.....	.....	22.26	.....	.....	.638	.....	.....
Averages, limits, and totals	2	2	[2] 16.7	19.88	.....	.....	1.98	.....	.....	17.90	.....	.....	.840	.....	.....
BACCHUS.															
Kelleys Island, Ohio	1	1	21.0	23.12	.....	.....	4.21	.....	.....	18.91	.....	.....	1.996	.....	.....
Middle Bass, Ohio	3	3	21.9	24.37	25.17	23.51	4.22	4.30	4.16	20.15	21.01	19.30	1.587	1.816	1.620
North Bass, Ohio	1	1	(1)	22.20	.....	.....	4.10	.....	.....	18.10	.....	.....	2.156	.....	.....
Sandusky, Ohio [3]	1	1	22.6	25.09	.....	.....	4.56	.....	.....	20.53	.....	.....	1.672	.....	.....
Brocton, N. Y	1	1	21.4	22.83	.....	.....	3.33	.....	.....	18.50	.....	.....	1.936	.....	.....
Averages, limits, and totals	7	7	[4] 21.8	23.76	25.17	23.51	4.12	4.30	4.16	19.64	21.01	19.30	1.852	1.816	1.620

[1] Brix not read.
[2] One sample.
[3] A. Schmidt, jr., & Bros.
[4] Only 6 samples.

Table II.—*Average, maximum, and minimum data for 1909, arranged by varieties and localities*—Continued.

Variety and locality.	Number of samples.	Number of growers.	Average Brix.	Total solids.			Sugar-free solids.			Sugar as invert.			Acid as tartaric.		
				Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.
Brighton.															
Put in Bay, Ohio........	1	1	18.9	20.62	.....	.....	2.25	.....	.....	18.37	.....	.....	.653	.....	.....
Venice, Ohio............	1	1	(1)	25.30	.....	.....	2.73	.....	.....	22.57	.....	.....	.611	.....	.....
Averages, limits, and totals.......	2	2	18.9	22.96	.....	.....	2.49	.....	.....	20.47	.....	.....	.632	.....	.....
Campbell's Early.															
Kelleys Island, Ohio.....	1	1	(1)	15.94	.....	.....	2.33	.....	.....	13.61	.....	.....	.784	.....	.....
Clinton.															
Brownhelm, Ohio.......	2	1	[2] 22.1	21.95	24.59	19.31	4.23	4.26	4.21	17.72	20.38	15.05	1.935	2.130	1.740
Huron, Ohio............	1	1	(1)	25.67	.....	.....	3.89	.....	.....	21.78	.....	.....	1.456	.....	.....
Middle Bass, Ohio.......	2	1	22.0	24.27	.....	.....	3.84	.....	.....	20.43	.....	.....	1.730	.....	.....
North Bass, Ohio........	2	2	(1)	22.43	21.41	20.44	3.56	3.66	3.47	17.36	17.75	16.97	1.755	1.808	1.702
Sandusky, Ohio [3]........	1	1	22.1	25.17	.....	.....	4.88	.....	.....	20.29	.....	.....	1.726	.....	.....
Sandusky, Ohio [4]........	1	1	20.0	21.78	.....	.....	3.87	.....	.....	17.91	.....	.....	1.628	.....	.....
Venice, Ohio............	4	2	[2] 18.7	18.92	19.97	17.83	4.05	4.26	3.82	14.87	15.76	14.01	1.568	1.932	1.309
North East, Pa..........	1	1	22.1	24.70	.....	.....	4.64	.....	.....	20.06	.....	.....	2.220	.....	.....
Bluff Point, N. Y.......	2	1	21.2	23.30	24.91	21.70	4.18	4.19	4.18	19.12	20.72	17.52	1.946	2.070	1.822
Brocton, N. Y..........	1	1	18.9	20.49	.....	.....	4.03	.....	.....	16.46	.....	.....	2.130	.....	.....
Averages, limits, and totals.......	17	12	20.9	22.07	24.91	17.83	4.08	4.26	3.47	17.80	20.72	14.01	1.777	1.808	1.309
Catawba.															
Brownhelm, Ohio.......	2	2	19.7	21.96	23.07	20.86	3.27	3.76	2.79	18.69	19.31	18.07	1.203	1.316	1.091
Danbury, Ohio..........	1	1	19.1	20.49	.....	.....	2.73	.....	.....	17.76	.....	.....	1.486	.....	.....
Euclid, Ohio............	1	1	(1)	19.63	.....	.....	2.87	.....	.....	16.76	.....	.....	1.249	.....	.....
Kelleys Island, Ohio.....	7	7	19.3	20.99	22.25	19.34	2.90	3.66	2.41	18.09	19.31	16.93	1.278	1.384	1.163
Middle Bass, Ohio.......	9	5	[5] 19.7	21.13	23.20	19.60	2.49	2.82	2.32	18.63	20.88	17.15	1.033	1.174	.881
North Bass, Ohio........	7	7	[6] 19.9	21.69	23.35	18.84	2.50	3.18	2.03	19.18	21.32	16.38	1.024	1.181	.844
Put in Bay, Ohio........	17	13	[7] 19.0	20.83	22.80	18.48	2.70	3.32	2.11	18.14	19.68	15.97	1.096	1.335	.960
Sandusky, Ohio:															
American Wine Co....	16	1	[8] 19.0	20.80	21.04	20.39	2.85	3.62	2.23	18.01	18.81	16.77	1.175	1.552	.945
Bass Island Vineyard Co..................	7	1	[9] 19.2	20.60	21.46	19.79	2.84	3.60	2.14	17.76	18.84	16.19	1.291	1.702	.866
Duroy & Haines.......	4	1	18.9	20.77	22.15	19.44	2.77	3.10	2.40	18.01	19.05	16.35	1.120	1.256	.968
Engels & Krudwig....	1	1	20.3	22.16	.....	.....	3.58	.....	.....	18.57	.....	.....	1.226	.....	.....
A. Schmidt, jr., & Bros................	3	1	17.2	18.27	19.00	17.09	2.15	2.17	2.13	16.12	16.86	14.92	1.113	1.181	1.058
Sweet Valley Wine Co.	3	1	18.0	19.18	21.15	18.01	2.33	2.47	2.16	16.86	18.68	15.85	1.190	1.238	1.163
Venice, Ohio............	6	5	[10] 19.3	20.37	23.13	18.92	2.82	3.22	2.25	17.55	20.11	15.73	1.207	2.432	1.058
North East, Pa..........	3	3	17.4	19.01	19.60	18.40	3.01	3.21	2.89	16.00	16.66	15.51	1.527	1.642	1.410
Brocton, N. Y..........	2	2	16.8	17.86	19.26	16.47	3.07	3.55	2.59	14.79	16.67	12.92	1.792	1.972	1.612
Ripley, N. Y...........	1	1	19.2	21.07	.....	.....	2.29	.....	.....	18.78	.....	.....	1.144	.....	.....
Sheridan, N. Y..........	1	1	17.9	19.42	.....	.....	2.20	.....	.....	17.22	.....	.....	(11)	.....	.....
Averages, limits, and totals.......	91	54	19.0	22.80	23.35	16.47	2.72	3.76	2.03	17.91	20.11	12.92	1.167	2.432	.844
Concord.															
Brownhelm, Ohio.......	3	3	[2] 17.8	17.58	18.97	16.68	2.48	2.86	1.90	15.10	17.07	14.01	.841	.930	.698
Catawba Island, Ohio...	1	1	16.9	17.75	.....	.....	2.03	.....	.....	15.72	.....	.....	.533	.....	.....
Danbury, Ohio..........	6	4	[2] 16.2	17.66	19.81	16.52	2.32	2.56	1.84	15.33	17.25	14.50	.672	.803	.555
Euclid, Ohio............	1	1	(1)	19.79	.....	.....	2.73	.....	.....	17.42	.....	.....	.626	.....	.....
Gypsum, Ohio...........	1	1	17.9	18.19	.....	.....	2.30	.....	.....	15.89	.....	.....	.533	.....	.....
Kelleys Island, Ohio.....	4	2	[9] 18.8	20.07	21.81	19.08	2.16	2.85	1.81	17.91	19.99	16.49	.629	.776	.525
Middle Bass, Ohio.......	4	4	16.5	18.04	19.84	16.99	2.08	2.59	1.84	15.98	18.00	14.40	[12] .720	.....	.....
Noble, Ohio.............	1	1	(1)	18.08	.....	.....	2.66	.....	.....	15.42	.....	.....	.634	.....	.....
North Bass, Ohio........	4	4	[2] 18.2	18.33	19.44	17.25	2.50	3.07	1.61	15.84	16.81	14.88	.752	1.050	.461

[1] Brix not read.
[2] One sample.
[3] Engels & Krudwig.
[4] A. Schmidt, jr., & Bros.
[5] Eight samples.
[6] Five samples.
[7] Sixteen samples.
[8] Twelve samples.
[9] Three samples.
[10] Four samples.
[11] Acid not made.
[12] One acid given.

TABLE II.—*Average, maximum, and minimum data for 1909, arranged by varieties and localities*—Continued.

Variety and locality.	Number of samples.	Number of growers.	Average Brix.	Total solids.			Sugar-free solids.			Sugar as invert.			Acid as tartaric.		
				Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.
CONCORD—Continued.															
Port Clinton, Ohio	3	3	16.6	17.00	17.20	16.60	1.96	2.25	1.80	15.04	15.37	14.80	0.611	0.660	0.566
Put in Bay, Ohio	2	2	18.3	19.46	20.49	18.43	1.97	1.99	1.95	17.49	18.50	16.48	.645	.690	.600
Sandusky, Ohio:															
Bass Island Vineyard Co.	1	....	17.3	18.32	.....	.....	2.55	.....	.....	15.77	.....	.....	.739	.....	.....
Engels & Krudwig	1	....	17.0	18.32	.....	.....	2.66	.....	.....	15.66	.....	.....	.713	.....	.....
Sweet Valley Wine Co.	1	....	(1)	15.50	.....	.....	1.90	.....	.....	13.60	.....	.....	.930	.....	.....
Jno. Dorn	1	....	16.7	17.80	.....	.....	1.96	.....	.....	15.84	.....	.....	.724	.....	.....
Venice, Ohio	12	11	[2]17.8	18.74	21.23	16.65	2.39	2.79	1.74	16.35	18.44	14.22	[3].742	.968	.521
North East, Pa.	9	9	17.0	18.19	19.94	16.23	2.02	2.37	1.77	16.17	17.85	14.22	.874	1.076	.716
Bluff Point, N. Y	3	3	17.9	18.82	19.86	17.62	1.81	1.91	1.71	17.00	18.04	15.91	.729	.975	.488
Brocton, N. Y	2	2	17.3	18.39	18.56	18.22	2.06	2.29	1.84	16.32	16.72	15.93	.847	1.046	.649
Hammondsport, N. Y	2	2	16.7	17.77	17.93	17.62	1.79	1.80	1.78	15.98	16.13	15.84	.613	.645	.581
Keuka, N. Y	3	3	16.3	17.07	17.85	16.23	1.88	1.92	1.81	15.20	15.95	14.42	.753	.814	.705
Penn Yan, N. Y	1	1	16.7	17.64	.....	.....	2.15	.....	.....	15.49	.....	.....	.716	.....	.....
Pulteney, N. Y	1	1	15.5	15.84	.....	.....	1.80	.....	.....	14.04	.....	.....	.615	.....	.....
Ripley, N. Y	3	3	16.8	17.82	19.05	16.47	1.91	2.03	1.83	15.91	17.18	14.64	[4].827	.915	.739
Sheridan, N. Y	1	1	(1)	17.90	.....	.....	1.99	.....	.....	15.91	.....	.....	.829	.....	.....
Wayne, N. Y	2	2	16.9	17.80	17.80	17.80	1.70	1.79	1.61	16.10	16.19	16.01	.690	.735	.645
Westfield, N. Y	3	3	16.3	17.31	18.06	16.23	2.14	2.41	2.00	15.17	16.06	14.22	.893	.990	.799
Averages, limits, and totals	76	67	17.0	18.14	21.81	16.23	2.15	3.07	1.61	15.99	19.99	14.01	.748	1.076	.461
CYNTHIANA.															
Put in Bay, Ohio	1	1	(1)	24.14	.....	.....	5.04	.....	.....	19.10	.....	.....	1.598	.....	.....
DELAWARE.															
Brownhelm, Ohio	1	1	24.2	26.70	.....	.....	2.22	.....	.....	24.28	.....	.....	.705	.....	.....
Danbury, Ohio	1	1	(1)	19.44	.....	.....	2.70	.....	.....	16.65	.....	.....	.908	.....	.....
Euclid, Ohio	1	1	(1)	20.89	.....	.....	2.74	.....	.....	18.15	.....	.....	.803	.....	.....
Kelleys Island, Ohio	2	1	[5]22.1	24.25	24.30	24.20	2.21	2.45	1.97	22.04	22.23	21.85	.765	.878	.653
Middle Bass, Ohio	7	7	(1)	22.81	24.99	21.02	2.00	2.13	1.89	20.81	23.04	18.89	[4].773	.818	.728
Noble, Ohio	1	1	(1)	24.04	.....	.....	2.59	.....	.....	21.45	.....	.....	.791	.....	.....
North Bass, Ohio	5	5	[5]22.7	23.31	25.07	22.78	2.25	3.21	2.19	21.47	23.34	20.50	.823	1.031	.641
Sandusky, Ohio:															
Sweet Valley Wine Co.	2	1	21.3	23.09	25.43	20.45	2.32	2.46	2.19	20.76	23.24	18.29	.735	.739	.731
Venice, Ohio	2	2	(1)	25.21	27.12	23.20	2.86	2.89	2.84	22.34	24.28	20.41	.943	1.031	.855
North East, Pa.	2	2	21.8	24.07	25.49	22.65	2.55	2.67	2.43	21.54	22.82	20.22	1.022	1.061	.983
Bluff Point, N. Y	2	2	20.7	22.46	22.72	22.20	2.34	2.89	1.80	20.11	20.92	19.31	.758	.788	.728
Brocton, N. Y	1	1	20.2	22.17	.....	.....	2.94	.....	.....	19.23	.....	.....	1.016	.....	.....
Hammondsport, N. Y	3	3	20.5	21.99	22.86	21.04	1.96	2.10	1.85	20.03	20.92	19.19	.765	.803	.716
Penn Yan, N. Y	1	1	21.6	23.88	.....	.....	2.84	.....	.....	21.04	.....	.....	.934	.....	.....
Pulteney, N. Y	1	1	20.5	21.75	.....	.....	2.05	.....	.....	19.70	.....	.....	.881	.....	.....
Ripley, N. Y	1	1	18.4	19.05	.....	.....	1.95	.....	.....	17.10	.....	.....	.788	.....	.....
Sheridan, N. Y	1	1	21.0	22.93	.....	.....	2.20	.....	.....	20.73	.....	.....	.806	.....	.....
Wayne, N. Y	1	1	20.9	22.75	.....	.....	2.13	.....	.....	20.62	.....	.....	.821	.....	....
Westfield, N. Y	1	1	17.3	18.56	.....	.....	2.36	.....	.....	16.20	.....	.....	.840	.....	.....
Averages, limits, and totals	36	34	20.9	22.97	25.49	20.45	2.36	3.21	1.80	20.61	24.28	19.19	.829	1.061	.641
DIANA.															
Kelleys Island, Ohio	1	1	20.1	22.07	.....	.....	2.17	.....	.....	19.90	.....	.....	.950	.....	.....
ELVIRA.															
Kelleys Island, Ohio	1	1	19.1	20.05	.....	.....	1.98	.....	.....	18.07	.....	.....	.765	.....	.....
Middle Bass, Ohio	4	4	(1)	16.08	17.85	14.64	2.25	2.45	1.98	13.82	15.87	12.40	.908	1.095	.656
North Bass, Ohio	2	2	(1)	16.41	18.32	14.51	2.51	3.25	2.78	13.40	15.07	11.13	1.277	1.354	1.200
Venice, Ohio	1	1	(1)	17.17	.....	.....	3.00	.....	.....	14.17	.....	.....	1.069	.....	.....
Averages, limits, and totals	8	8	19.1	16.66	18.32	14.51	2.50	3.25	1.98	14.29	15.87	11.13	1.002	1.354	.656

[1] Brix not read.
[2] Four samples.
[3] Ten acids given.
[4] Two acids given.
[5] On one sample.

TABLE II.—*Average, maximum, and minimum data for 1909, arranged by varieties and localities*—Continued.

Variety and locality.	Number of samples.	Number of growers.	Average Brix.	Total solids.			Sugar-free solids.			Sugar as invert.			Acid as tartaric.		
				Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.
ETTA.															
North Bass, Ohio........	1	1	13.9	14.20	.....	.....	2.96	.....	.....	11.24	.....	.....	1.369	.....	.....
GREENS EARLY.															
Kelleys Island, Ohio	1	1	(1)	18.45	.....	.....	4.24	.....	.....	14.21	.....	.....	.758	.....	.....
GUT EDEL.															
Kelleys Island, Ohio	1	1	21.9	23.77	.....	.....	3.57	.....	.....	20.20	.....	.....	1.144	.....	.....
HAYS.															
Put in Bay, Ohio........	1	1	(1)	20.94	.....	.....	2.79	.....	.....	18.45	.....	.....	.893	.....	.....
HARTFORD.															
Middle Bass, Ohio.......	1	1	19.1	19.60	.....	.....	2.04	.....	.....	17.56	.....	.....	(2)	.....	.....
IONA.															
Kelleys Island, Ohio	2	2	19.9	21.90	22.36	21.44	2.27	2.67	1.87	19.63	20.49	18.77	.806	.855	.758
Put in Bay, Ohio........	1	1	19.3	21.83	.....	.....	3.02	.....	.....	18.81	.....	.....	.851	.....	.....
Brocton, N. Y...........	1	1	21.0	23.14	.....	.....	2.07	.....	.....	21.07	.....	.....	.881	.....	.....
Averages, limits, and totals.......	4	4	20.0	22.19	22.36	21.44	2.41	2.67	1.87	19.78	20.49	18.77	.836	.855	.758
ISABELLA.															
Euclid, Ohio............	1	1	(1)	15.55	.....	.....	2.22	.....	.....	13.33	.....	.....	.821	.....	.....
Marblehead, Ohio........	1	1	(1)	13.13	.....	.....	2.21	.....	.....	10.92	.....	.....	1.084	.....	.....
North East, Pa..........	1	1	14.9	15.94	.....	.....	2.44	.....	.....	13.50	.....	.....	1.140	.....	.....
Bluff Point, N. Y.......	2	2	16.6	17.60	17.67	17.54	2.09	2.16	2.02	15.51	15.52	15.51	.872	.874	.870
Brocton, N. Y...........	1	1	16.2	17.25	.....	.....	2.57	.....	.....	14.68	.....	.....	1.249	.....	.....
Averages, limits, and totals.......	6	6	16.1	16.18	17.67	17.54	2.27	2.16	2.02	13.91	15.52	15.51	1.008	.874	.870
IVES.															
Brownhelm, Ohio........	3	2	[3] 17.5	18.73	19.44	17.64	3.05	3.45	2.53	15.68	15.99	15.11	.935	1.118	.720
Huron, Ohio............	2	2	[3] 17.6	18.04	19.29	16.80	2.71	2.97	2.45	15.33	16.32	14.35	.637	.656	.619
Kelleys Island, Ohio....	1	1	(1)	16.15	.....	.....	2.78	.....	.....	13.37	.....	.....	.896	.....	.....
Middle Bass, Ohio.......	2	2	16.7	17.78	19.00	16.57	2.53	2.79	2.28	15.25	16.21	14.29	.716	.784	.649
North Bass, Ohio........	2	2	[3] 14.3	14.79	15.03	14.56	2.20	2.30	2.11	12.59	12.73	12.45	.742	.765	.720
Sandusky, Ohio:															
Engels & Krudwig....	3	1	16.7	17.94	18.69	16.62	2.63	2.79	2.50	15.31	16.19	14.03	.651	.743	.525
Sweet Valley Wine Co.	2	1	17.0	18.66	18.71	18.61	3.29	3.62	2.96	15.37	15.65	15.09	.744	.780	.709
John Dorn............	1	1	18.9	20.81	.....	.....	3.83	.....	.....	16.98	.....	.....	.788	.....	.....
A. Schmidt, jr., & Bros................	1	1	18.8	20.28	.....	.....	3.11	.....	.....	17.17	.....	.....	.833	.....	.....
Put in Bay, Ohio	2	2	16.9	18.26	18.58	17.95	2.84	3.19	2.50	15.42	16.08	14.76	.697	.735	.559
Venice, Ohio............	6	5	[4] 16.6	17.15	18.50	15.22	2.59	2.92	1.81	14.56	15.73	13.41	[5] .815	.994	.716
Vermilion, Ohio.........	3	2	[3] 17.4	17.30	17.75	16.47	2.30	2.73	2.04	15.00	15.71	13.74	.775	.844	.664
North East, Pa..........	1	1	12.6	13.39	.....	.....	2.22	.....	.....	11.17	.....	.....	.776	.....	.....
Hammondsport, N. Y...	1	1	14.9	15.35	.....	.....	1.88	.....	.....	13.47	.....	.....	.683	.....	.....
Pulteney, N. Y..........	1	1	12.3	12.92	.....	.....	2.16	.....	.....	10.76	.....	.....	.904	.....	.....
Averages, limits, and totals.......	31	25	16.4	17.39	19.44	14.56	2.67	3.62	1.81	14.72	16.32	12.45	.768	1.118	.619
MARTHA.															
Brocton, N. Y...........	1	1	17.2	18.06	.....	.....	1.99	.....	.....	16.07	.....	.....	.840	.....	.....

[1] Brix not read.
[2] Acid not made.
[3] One sample.
[4] Two samples.
[5] Four acids given.

TABLE II.—*Average, maximum, and minimum data for 1909, arranged by varieties and localities*—Continued.

Variety and locality.	Number of samples.	Number of growers.	Average Brix.	Total solids.			Sugar-free solids.			Sugar as invert.			Acid as tartaric.		
				Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.
MARION.															
North Bass, Ohio	1	1	23.1	25.17	.....	.....	4.40	.....	.....	20.77	.....	.....	1.718	.....	.....
MONTEFIORE.															
Kelleys Island, Ohio	2	1	[1] 18.0	21.21	23.35	19.08	2.68	3.22	2.16	18.52	20.13	16.92	.771	1.073	.469
Put in Bay, Ohio	1	1	(2)	24.54	.....	.....	3.27	.....	.....	21.27	.....	.....	.780	.....	.....
Venice, Ohio	1	1	(2)	24.54	.....	.....	3.04	.....	.....	21.51	.....	.....	.645	.....	.....
Averages, limits, and totals	4	3	18.0	22.88	23.35	19.08	2.92	3.22	2.16	19.96	20.13	16.92	.842	1.073	.469
NIAGARA.															
Brownhelm, Ohio	1	1	(2)	21.36	.....	.....	2.99	.....	.....	18.37	.....	.....	.718	.....	.....
Danbury, Ohio	1	1	(2)	15.63	.....	.....	2.66	.....	.....	12.97	.....	.....	.585	.....	.....
Euclid. Ohio	1	1	(2)	15.32	.....	.....	2.58	.....	.....	12.74	.....	.....	.551	.....	.....
Kelleys Island, Ohio	1	1	16.9	18.30	.....	.....	1.80	.....	.....	16.50	.....	.....	.585	.....	.....
Middle Bass, Ohio	1	1	(2)	18.74	.....	.....	3.17	.....	.....	15.57	.....	.....	.675	.....	.....
North Bass, Ohio	1	1	(2)	15.61	.....	.....	2.68	.....	.....	12.93	.....	.....	.581	.....	.....
North East, Pa	3	3	18.3	19.57	20.02	19.31	1.92	2.06	1.83	17.65	17.96	17.43	.781	.829	.746
Bluff Point, N. Y	2	1	16.1	16.74	17.93	15.55	1.48	1.80	1.16	15.76	16.13	14.39	.501	.664	.338
Brocton, N. Y	1	1	18.3	20.12	.....	.....	2.00	.....	.....	18.12	.....	.....	.690	.....	.....
Ripley, N. Y	3	3	16.6	17.72	18.16	17.33	1.84	1.93	1.80	15.88	16.23	15.53	[3] .665	.731	.599
Westfield, N. Y	1	1	15.7	16.44	.....	.....	3.30	.....	.....	13.14	.....	.....	.780	.....	.....
Averages, limits, and totals	16	15	17.1	17.93	20.02	15.55	2.22	2.06	1.16	15.72	17.96	14.39	.615	.829	.338
NORTON.															
Euclid, Ohio	1	1	(2)	25.85	.....	.....	3.34	.....	.....	22.51	.....	.....	1.456	.....	.....
Kelleys Island, Ohio	1	1	19.5	20.94	.....	.....	3.25	.....	.....	17.69	.....	.....	1.770	.....	.....
Middle Bass, Ohio	2	2	21.3	22.29	22.88	21.70	4.49	4.79	4.18	17.80	18.70	16.91	1.896	1.912	1.880
North Bass, Ohio	2	2	(2)	21.84	21.94	21.75	4.46	4.72	4.21	17.38	17.54	17.22	1.718	1.740	1.696
Put in Bay, Ohio	2	1	[1] 22.0	23.79	23.91	23.67	4.21	4.23	4.19	19.58	19.68	19.48	1.864	1.912	1.816
Sandusky, Ohio:															
Engels & Krudwig	1	1	25.4	27.96	.....	.....	6.13	.....	.....	21.83	.....	.....	1.996	.....	.....
Venice, Ohio	2	2	24.2	26.70	26.78	26.63	5.20	5.51	4.90	21.50	21.72	21.27	1.917	2.078	1.756
Averages, limits, and totals	11	10	22.5	24.00	26.78	21.70	4.49	5.51	4.18	19.50	21.72	16.91	1.812	2.078	1.696
POCKLINGTON.															
Put in Bay, Ohio	1	1	17.8	19.26	.....	.....	2.70	.....	.....	16.80	.....	.....	.926	.....	.....
Venice, Ohio	1	1	17.9	19.55	.....	.....	2.51	.....	.....	17.26	.....	.....	.656	.....	.....
Ripley, N. Y	1	1	(2)	18.19	.....	.....	1.72	.....	.....	16.15	.....	.....	.518	.....	.....
Averages, limits, and totals	3	3	17.8	19.00	.....	.....	2.31	.....	.....	16.85	.....	.....	.700	.....	.....
RIESLING.															
Brownhelm, Ohio	1	1	(2)	21.88	.....	.....	2.94	.....	.....	18.94	.....	.....	1.181	.....	.....
Kelleys Island, Ohio	2	2	19.8	21.61	22.65	20.57	2.42	2.47	2.38	19.18	20.18	18.19	.750	.825	.675
Averages, limits, and totals	3	3	19.8	21.70	22.65	20.57	2.59	2.47	2.38	19.13	20.18	18.19	.894	.825	.675
ROGERS.															
Brocton, N. Y	1	1	18.6	20.23	.....	.....	2.13	.....	.....	18.10	.....	.....	1.035	.....	.....
SALEM.															
North Bass, Ohio	1	1	(2)	19.21	.....	.....	1.60	.....	.....	17.61	.....	.....	.510	.....	.....

[1] One sample.

[2] Brix not read.

[3] Two acids given.

TABLE II.—*Average, maximum, and minimum data for 1909, arranged by varieties and localities*—Continued.

Variety and locality.	Number of samples.	Number of growers.	Average Brix.	Total solids.			Sugar-free solids.			Sugar as invert.			Acid as tartaric.		
				Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.
SCHRAIDT.															
Middle Bass, Ohio.......	2	2	20.2	22.37	22.88	21.86	4.95	5.74	4.17	17.41	17.69	17.14	1.853	2.040	1.666
North Bass, Ohio........	1	1	(1)	21.70	.....	.....	4.02	.....	.....	17.68	.....	.....	1.726	.....	.....
Averages, limits, and totals.......	3	3	20.2	22.15	22.88	21.86	4.64	5.74	4.17	17.50	17.69	17.14	1.823	2.040	1.666
UNKNOWN GRAPES.															
Brownhelm, Ohio........	1	1	20.1	22.57	.....	.....	3.49	.....	.....	18.08	.....	.....	.754	.....	.....
Kelleys Island, Ohio.....	2	2	(1)	21.98	23.22	20.75	2.56	2.79	2.34	19.42	20.43	18.41	.797	.938	.656
Middle Bass, Ohio.......	2	2	17.0	18.42	20.36	16.49	2.52	3.05	1.99	15.91	18.37	13.44	.908	1.301	.514
North Bass, Ohio........	1	1	20.3	21.10	.....	.....	3.71	.....	.....	17.39	.....	.....	1.365	.....	.....
Sandusky, Ohio:															
A. Schmidt, jr., & Bros.	1	....	19.7	.....	.....	.....	.....	.....	.....	.....	.....	.....	.806	.....	.....
Venice, Ohio............	1	1	(1)	18.16	.....	.....	2.60	.....	.....	15.93	.....	.....	.679	.....	.....
Vermilion, Ohio..........	1	1	21.5	23.83	.....	.....	2.65	.....	.....	21.21	.....	.....	.979	.....	.....
Averages, limits, and totals.......	9	8	19.1	20.81	23.22	16.49	2.83	3.05	1.99	18.03	20.43	13.44	.898	1.310	.514
WILDER.															
Put in Bay, Ohio........	1	1	16.1	16.96	.....	.....	2.52	.....	.....	14.44	.....	.....	1.020	.....	.....
EARLY WORDEN.															
Put in Bay, Ohio........	1	1	(1)	19.68	.....	.....	2.90	.....	.....	17.02	.....	.....	.780	.....	.....
Venice, Ohio............	1	1	17.8	19.31	.....	.....	2.04	.....	.....	17.47	.....	.....	.638	.....	.....
Averages, limits, and totals.......	2	2	17.8	19.49	.....	.....	2.47	.....	.....	17.24	.....	.....	.709	.....	.....
WOODRUFF RED.															
Middle Bass, Ohio.......	1	1	(1)	17.27	.....	.....	2.93	.....	.....	14.34	.....	.....	.409	.....	.....

[1] Brix not read.

DATA FOR 1910.

TABLE III.—*Average, maximum, and minimum data for 1910, arranged by varieties and localities.* (*Analyses by Eoff and Treuthardt.*)

[Results expressed in grams per 100 cc of expressed juice.]

Variety and locality.	Number of samples.	Number of growers.	Brix.	Total solids.			Sugar-free solids.			Sugar as invert.			Acid as tartaric.		
				Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.
AGAWAM.															
Fredonia, N. Y	2	2	16.5	17.88	18.40	17.35	3.23	3.47	2.99	14.65	14.93	14.36	1.463	1.516	1.410
Geneva, N. Y	1	1	17.9	19.16	.....	.....	2.62	.....	.....	16.54	.....	.....	1.106	.....	.....
Averages, limits, and totals	3	3	17.0	18.30	18.40	17.35	3.03	3.47	2.99	15.28	14.93	14.36	1.344	1.516	1.410
AMBER QUEEN.															
Geneva, N. Y	1	1	17.6	18.63	.....	.....	2.61	.....	.....	16.02	.....	.....	1.436	.....	.....
AMERICA.															
Geneva, N. Y	1	1	18.6	19.65	.....	.....	2.71	.....	.....	16.94	.....	.....	1.043	.....	.....
AMINIA.															
Geneva, N. Y	1	1	17.5	18.50	.....	.....	1.82	.....	.....	16.68	.....	.....	.821	....	.....
ADIRONDACK.															
Geneva, N. Y	1	1	11.9	12.35	.....	.....	2.16	.....	.....	10.19	.....	.....	.911	.....	.....
BACCHUS.															
Kelleys Island, Ohio	1	1	23.1	25.38	.....	.....	3.27	.....	.....	22.11	.....	.....	1.714	.....	.....
North Bass, Ohio	1	1	24.5	16.07	.....	.....	3.34	.....	.....	12.73	.....	.....	1.826	.....	.....
Averages, limits, and totals	2	2	23.8	20.73	.....	.....	3.31	.....	.....	17.42	.....	.....	1.770	.....	.....
BARRY.															
Geneva, N. Y	1	1	14.0	14.59	.....	.....	2.47	.....	.....	12.12	.....	.....	1.159	.....	.....
BELL.															
Geneva, N. Y	1	1	20.1	21.78	.....	.....	3.00	.....	.....	18.78	.....	.....	1.114	.....	.....
BIG EXTRA 19.															
Geneva, N. Y	1	1	16.5	17.01	.....	.....	3.10	.....	.....	13.91	.....	.....	1.466	.....	.....
BLACK EAGLE.															
Geneva, N. Y	2	1	18.6	19.88	21.88	17.88	2.31	2.40	2.22	17.57	19.66	15.48	1.011	1.121	.900
BRIGHTON.															
Put in Bay, Ohio	1	1	17.8	18.90	.....	.....	2.50	.....	.....	16.40	.....	.....	.982	.....	.....
Venice, Ohio	3	1	[1]21.8	22.24	24.14	19.16	2.38	2.83	1.93	19.87	21.50	16.79	.713	.840	.630
Fredonia, N. Y	1	1	17.1	18.40	.....	.....	2.19	.....	.....	16.21	.....	.....	.900	.....	.....
Geneva, N. Y	1	1	18.7	19.92	.....	.....	2.19	.....	.....	17.73	.....	.....	.803	.....	.....
Pulteney, N. Y	1	1	21.3	23.07	.....	.....	2.33	.....	.....	20.74	.....	.....	.934	.....	.....
Averages, limits, and totals	7	5	19.3	21.00	24.14	19.16	2.33	2.83	1.93	18.67	21.50	16.79	.822	.840	.630
BROWN.															
Geneva, N. Y	1	1	15.8	16.36	.....	.....	2.72	.....	.....	13.64	.....	.....	1.106	.....	.....
BUTLER NO. 1.															
Geneva, N. Y	1	1	17.6	19.18	.....	.....	2.15	.....	.....	17.03	.....	.....	.833	.....	.....

[1] One sample.

TABLE III.—*Average, maximum, and minimum data for 1910, arranged by varieties and localities*—Continued.

Variety and locality.	Number of samples.	Number of growers.	Brix.	Total solids.			Sugar-free solids.			Sugar as invert.			Acid as tartaric.		
				Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.
CAMPBELL'S EARLY.															
Kelleys Island, Ohio.....	1	1	15.7	20.34	.....	.....	1.84	.....	.....	18.50	.....	.....	0.611	.....	.....
Middle Bass, Ohio.......	1	1	17.3	18.84	.....	.....	1.97	.....	.....	16.87	.....	.....	.641	.....	.....
Put in Bay, Ohio........	1	1	15.2	15.74	.....	.....	2.28	.....	.....	13.46	.....	.....	1.042	.....	.....
Fredonia, N. Y..........	1	1	15.4	16.31	.....	.....	2.90	.....	.....	13.41	.....	.....	1.241	.....	.....
Averages, limits, and totals.......	4	4	16.7	17.81	.....	.....	2.25	.....	.....	15.56	.....	.....	.884	.....	.....
CARMAN.															
Geneva, N. Y...........	1	1	16.1	16.73	.....	.....	3.44	.....	.....	13.29	.....	.....	1.654	.....	.....
CATAWBA.															
Kelleys Island, Ohio.....	14	11	21.0	23.14	25.17	21.46	2.30	2.57	2.02	20.84	22.70	19.31	.999	1.170	0.840
Middle Bass, Ohio.......	28	22	21.1	23.39	24.99	21.78	2.46	3.02	1.86	20.93	22.55	19.25	1.063	1.305	.754
North Bass, Ohio........	10	9	21.4	23.84	24.80	22.83	2.32	2.68	1.88	21.52	22.87	20.35	1.058	1.226	.795
Put in Bay, Ohio........	10	9	20.7	22.82	24.57	20.23	2.52	2.73	2.27	20.30	22.01	17.96	1.192	1.335	1.095
Venice, Ohio............	5	3	[1]21.3	23.09	24.59	20.89	2.35	2.54	2.10	20.74	22.21	18.65	1.045	1.144	.938
Vermilion, Ohio	1	1	21.7	24.17	.....	.....	2.51	.....	.....	21.66	.....	.....	1.091	.....	.....
Sandusky, Ohio:															
Cook's Wine Cellar....	16	1	20.7	22.85	23.41	21.99	2.62	3.56	2.18	20.23	20.88	18.43	1.091	1.219	.893
Bluff Point, N. Y.......	1	1	20.0	21.75	.....	.....	2.80	.....	.....	18.95	.....	.....	1.444	.....	.....
Jerusalem, N. Y.........	1	1	19.6	21.28	.....	.....	2.95	.....	.....	18.33	.....	.....	1.606	.....	.....
Pulteney, N. Y..........	1	1	21.3	23.38	.....	.....	2.60	.....	.....	20.78	.....	.....	1.230	.....	.....
Urbana, N. Y............	4	4	20.3	22.31	23.38	20.81	2.71	2.92	2.30	19.60	20.65	17.89	1.286	1.530	.994
North East, Pa.:															
South Shore Wine Co..	2	1	18.0	19.25	19.26	19.23	3.15	3.49	2.81	16.20	16.42	15.77	1.729	1.996	1.462
Averages, limits, and totals.......	93	64	20.0	23.07	25.17	19.23	2.50	3.56	1.86	20.51	22.87	15.77	1.097	1.996	.754
CHAMPION.															
Geneva, N. Y...........	1	1	13.2	14.30	.....	.....	2.40	.....	.....	11.90	.....	.....	.915	.....	.....
CHAUTAUQUA.															
Geneva, N. Y...........	1	1	15.2	15.84	.....	.....	2.34	.....	.....	13.50	.....	.....	1.043	.....	.....
CLEVNER 7-12.															
Geneva, N. Y...........	1	1	17.8	18.71	.....	.....	3.75	.....	.....	14.96	.....	.....	1.196	.....	.....
CLINTON.															
North Bass, Ohio........	1	1	23.5	26.01	.....	.....	3.68	.....	.....	22.33	.....	.....	1.520	.....	.....
Venice, Ohio............	2	2[2]	23.4	23.89	25.91	21.86	2.85	4.69	3.00	20.04	22.91	17.17	2.117	2.588	1.646
Geneva, N. Y...........	1	1	19.5	20.78	.....	.....	4.60	.....	.....	16.18	.....	.....	2.272	.....	.....
Pulteney, N. Y..........	1	1	24.1	26.46	.....	.....	3.26	.....	.....	23.20	.....	.....	1.726	.....	.....
North East, Pa.:															
South Shore Wine Co..	2	....	20.8	22.08	23.14	21.02	3.72	3.94	3.50	18.36	19.64	17.08	2.260	2.494	2.026
Averages, limits, and totals.......	7	5	22.3	23.60	.....	.....	3.81	.....	.....	19.79	.....	.....	2.039	.....	.....
CLOETA 19-2.															
Geneva, N. Y...........	1	1	([3])	18.92	.....	.....	2.90	.....	.....	16.02	.....	.....	1.088	.....	.....
COLERAIN.															
Geneva, N. Y...........	1	1	16.5	17.48	.....	.....	4.72	.....	.....	12.76	.....	.....	.870	.....	.....
CORBY.															
Geneva, N. Y...........	1	1	15.3	15.92	.....	.....	2.15	.....	.....	13.77	.....	.....	.938	.....	.....

[1] Four samples. [2] One sample. [3] Brix not read.

TABLE III.—*Average, maximum, and minimum data for 1910, arranged by varieties and localities*—Continued.

Variety and locality.	Number of samples.	Number of growers.	Brix.	Total solids.			Sugar-free solids.			Sugar as invert.			Acid as tartaric.		
				Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.
CONCORD.															
Kelleys Island, Ohio	1	1	19.0	20.81	.....	.....	2.29	.....	.....	18.52	.....	.....	0.623	.....	.....
Middle Bass, Ohio	19	19	[1] 18.4	20.84	21.73	17.01	2.44	3.16	1.80	17.41	19.23	16.03	.801	1.069	0.611
North Bass, Ohio	3	1	19.6	21.90	22.30	21.20	2.27	2.59	2.03	19.63	20.17	19.01	.650	.671	.608
Venice, Ohio	6	5	[2] 20.5	22.43	23.43	21.59	2.65	3.50	1.96	19.76	20.37	18.88	.679	.836	.536
Vermilion, Ohio	1	1	17.3	18.19	.....	.....	2.52	.....	.....	15.67	.....	.....	.840	.....	.....
Brocton, N. Y	8	1	18.7	20.50	21.36	19.60	2.09	2.63	1.87	18.16	19.49	17.17	.786	.983	.604
Bluff Point, N. Y	1	1	16.5	17.77	.....	.....	2.59	.....	.....	15.18	.....	.....	.926	.....	.....
Fredonia, N. Y	11	1	16.5	17.69	19.37	15.16	2.41	2.65	2.03	15.10	16.72	12.67	.925	1.009	.795
Geneva, N. Y	1	1	14.7	15.42	.....	.....	2.46	.....	.....	12.96	.....	.....	1.050	.....	.....
Hammondsport, N. Y	2	2	16.5	17.71	18.66	16.75	2.53	2.56	2.49	15.18	16.10	14.26	.986	1.013	.969
Jerusalem, N. Y	1	1	18.6	20.15	.....	.....	2.25	.....	.....	17.90	.....	.....	.731	.....	.....
Pulteney, N. Y	1	1	18.7	19.60	.....	.....	2.41	.....	.....	17.19	.....	.....	.979	.....	.....
Silver Creek, N. Y	2	1	15.9	17.19	18.40	15.97	2.41	2.61	2.21	14.78	16.19	13.36	1.030	1.110	.949
Urbana, N. Y.:															
Urbana Wine Co	2	1	16.8	17.66	18.45	16.86	2.25	2.28	2.21	15.41	16.17	14.65	.974	.979	.968
North East, Pa	23	9	17.7	19.04	23.01	12.90	2.45	2.76	2.17	16.58	20.48	10.41	.905	1.136	.671
Lawton, Mich	6	2	18.9	20.37	20.99	19.13	2.40	2.66	2.21	17.97	18.75	16.81	.761	.945	.660
Averages, limits, and totals	88	48	18.0	19.42	23.43	12.90	2.40	3.50	1.80	17.05	20.48	10.41	.834	1.069	.536
CREVELING.															
Geneva, N. Y	1	1	15.4	16.18	.....	.....	2.25	.....	.....	13.93	.....	.....	1.009	.....	.....
CROTON 11-4.															
Geneva, N. Y	1	1	13.4	15.07	.....	.....	3.23	.....	.....	11.84	.....	.....	.788	.....	.....
DAISY 7-2.															
Geneva, N. Y	1	1	18.0	19.29	.....	.....	2.34	.....	.....	16.95	.....	.....	.653	.....	.....
DELAGO.															
Geneva, N. Y	1	1	18.6	19.86	.....	.....	2.61	.....	.....	17.25	.....	.....	1.009	.....	.....
DELAWARE.															
Kelleys Island, Ohio	1	1	24.7	27.51	.....	.....	2.52	.....	.....	24.99	.....	.....	.641	.....	.....
Middle Bass, Ohio	6	6	[3] 23.6	26.51	27.62	24.46	2.41	2.72	2.15	23.76	25.47	22.01	.841	.909	.765
North Bass, Ohio	1	1	23.5	26.25	.....	.....	1.68	.....	.....	24.57	.....	.....	.724	.....	.....
Venice, Ohio	3	3	[4] 24.3	26.77	27.54	25.30	2.48	2.92	2.21	24.29	25.24	23.09	.773	.881	.713
Bluff Point, N. Y	1	1	20.5	22.96	.....	.....	3.14	.....	.....	19.82	.....	.....	1.013	.....	.....
Fredonia, N. Y	3	1	18.8	20.42	21.99	19.63	2.95	3.21	2.75	17.47	19.24	16.42	1.229	1.312	1.076
Geneva, N. Y	1	1	18.8	20.15	.....	.....	2.41	.....	.....	17.74	.....	.....	1.166	.....	.....
Hammondsport, N. Y	3	3	21.0	22.87	23.12	22.49	2.46	2.62	2.37	20.41	20.60	20.12	.866	.945	.743
Pulteney, N. Y	3	2	20.4	21.97	23.96	19.86	2.38	2.52	2.26	19.59	21.70	17.50	.968	1.020	.919
Rheims, N. Y	1	1	20.1	21.52	.....	.....	2.41	.....	.....	19.11	.....	.....	1.020	.....	.....
Urbana, N. Y.:															
Urbana Wine Co	2	1	22.4	24.41	24.91	23.91	2.26	2.31	2.20	22.16	22.71	21.60	.857	.859	.855
New York State:															
Name lost	1	1	20.0	21.41	.....	.....	2.42	.....	.....	18.99	.....	.....	1.001	.....	.....
North East, Pa	2	1	20.8	22.38	22.72	22.04	2.61	2.71	2.51	19.77	20.01	19.53	.981	.994	.968
Lawton, Mich	1	1	22.6	25.20	.....	.....	2.54	.....	.....	22.66	.....	.....	.945	.....	.....
Averages, limits, and totals	29	24	21.8	23.92	27.62	19.63	2.49	3.21	2.15	21.36	25.47	16.42	.922	1.312	.713
DIAMOND.															
Geneva, N. Y	1	1	17.9	19.03	.....	.....	2.45	.....	.....	16.58	.....	.....	.998	.....	.....

[1] Eighteen samples.

[2] Four samples.

[3] Nine samples.

[4] Three samples.

TABLE III.—*Average, maximum, and minimum data for 1910, arranged by varieties and localities*—Continued.

Variety and locality.	Number of samples.	Number of growers.	Brix.	Total solids.			Sugar-free solids.			Sugar as invert.			Acid as tartaric.		
				Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.
DIANA.															
North Bass, Ohio	1	1	21.5	23.70	.....	.....	2.35	.....	.....	21.35	.....	.....	0.728	.....	.....
Geneva, N. Y	1	1	19.6	21.23	.....	.....	2.66	.....	.....	18.67	.....	.....	1.200	.....	.....
Pulteney, N. Y	1	1	20.6	22.59	.....	.....	2.41	.....	.....	20.18	.....	.....	.923	.....	.....
Hammondsport, N. Y	1	1	21.3	23.57	.....	.....	2.68	.....	.....	20.89	.....	.....	1.009	.....	.....
Averages, limits, and totals	4	4	20.8	22.80	.....	.....	2.53	.....	.....	20.27	.....	.....	.965	.....	.....
DUCHESS.															
Geneva, N. Y	1	1	18.0	19.63	.....	.....	2.33	.....	.....	17.30	.....	.....	.750	.....	.....
Pulteney, N. Y	1	1	18.9	20.34	.....	.....	1.82	.....	.....	18.52	.....	.....	.638	.....	.....
Rheims, N. Y	1	1	17.8	18.87	.....	.....	2.24	.....	.....	16.63	.....	.....	.814	.....	.....
Averages, limits, and totals	3	3	18.3	19.61	.....	.....	2.13	.....	.....	17.48	.....	.....	.734	.....	.....
EARLY DAWN.															
Geneva, N. Y	1	1	17.6	18.56	.....	.....	2.34	.....	.....	16.22	.....	.....	1.065	.....	.....
EARLY GOLDEN 5-3.															
Geneva, N. Y	1	1	19.6	20.89	.....	.....	2.39	.....	.....	18.50	.....	.....	.848	.....	.....
EARLY VICTOR.															
Fredonia, N. Y	1	1	16.6	17.59	.....	.....	.....	.....	.....	8.07	.....	.....	.923	.....	.....
ELDORADO.															
Geneva, N. Y	1	1	20.3	22.02	.....	.....	2.39	.....	.....	19.63	.....	.....	.784	.....	.....
ELVIRA.															
Kelleys Island, Ohio	1	1	20.6	22.57	.....	.....	2.19	.....	.....	20.38	.....	.....	.754	.....	.....
Middle Bass, Ohio	1	1	16.5	17.14	.....	.....	2.72	.....	.....	14.42	.....	.....	1.050	.....	.....
North Bass, Ohio	1	1	17.4	18.92	.....	.....	1.46	.....	.....	17.46	.....	.....	.938	.....	.....
Venice, Ohio	1	1	17.8	19.05	.....	.....	2.68	.....	.....	16.37	.....	.....	.908	.....	.....
Hammondsport, N. Y	1	1	15.3	16.13	.....	.....	2.47	.....	.....	13.66	.....	.....	1.140	.....	.....
Pulteney, N. Y	1	1	16.1	16.70	.....	.....	2.50	.....	.....	14.20	.....	.....	1.264	.....	.....
Rheims, N. Y	1	1	15.5	15.79	.....	.....	2.78	.....	.....	13.01	.....	.....	1.462	.....	.....
Urbana, N. Y	1	1	17.4	18.74	.....	.....	2.69	.....	.....	16.05	.....	.....	1.144	.....	.....
Averages, limits, and totals	8	8	17.1	18.13	.....	.....	2.43	.....	.....	15.69	.....	.....	1.083	.....	.....
EMPIRE STATE.															
Put in Bay, Ohio	1	1	15.5	16.10	.....	.....	1.86	.....	.....	14.24	.....	.....	.848	.....	.....
Geneva, N. Y	1	1	18.7	20.18	.....	.....	2.23	.....	.....	17.95	.....	.....	.559	.....	.....
Averages, limits, and totals	2	2	17.1	18.14	.....	.....	2.05	.....	.....	16.10	.....	.....	.704	.....	.....
EUMELAN.															
North Bass, Ohio	1	1	14.9	15.58	.....	.....	2.04	.....	.....	13.54	.....	.....	.930	.....	.....
Geneva, N. Y	1	1	14.8	15.89	.....	.....	2.39	.....	.....	13.50	.....	.....	.964	.....	.....
Rheims, N. Y	1	1	19.8	21.20	.....	.....	2.15	.....	.....	18.87	.....	.....	.983	.....	.....
Urbana, N. Y.: Urbana Wine Co	2	1	20.2	21.58	21.75	21.41	2.17	2.20	2.13	19.42	19.62	19.21	.949	0.994	0.904
Averages, limits, and totals	5	4	18.0	19.13	21.75	21.41	2.18	2.20	2.13	16.95	19.62	19.21	.955	.994	.904
GOFF.															
Geneva, N. Y	2	1	16.8	18.07	21.67	14.46	2.43	2.51	2.35	15.64	19.82	11.95	.895	.964	.825

TABLE III.—*Average, maximum, and minimum data for 1910, arranged by varieties and localities*—Continued.

Variety and locality.	Number of samples.	Number of growers.	Brix.	Total solids.			Sugar-free solids.			Sugar as invert.			Acid as tartaric.		
				Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.
Gold Dust.															
Geneva, N. Y	1	1	17.1	18.24	.....	.....	2.53	.....	.....	15.71	.....	.....	0.694	.....	.....
Golden.															
Geneva, N. Y	1	1	17.4	18.69	.....	.....	1.65	.....	.....	17.04	.....	.....	.420	.....	.....
Glenfeld.															
Geneva, N. Y	1	1	16.5	17.25	.....	.....	2.69	.....	.....	14.56	.....	.....	1.106	.....	.....
Gut Edel.															
Kelleys Island, Ohio	1	1	21.5	23.20	.....	.....	2.84	.....	.....	20.36	.....	.....	1.350	.....	.....
Hayes.															
Put in Bay, Ohio	1	1	16.8	17.72	.....	.....	2.56	.....	.....	15.16	.....	.....	1.118	.....	.....
Hartford.															
Geneva, N. Y	1	1	19.3	20.83	.....	.....	2.67	.....	.....	18.16	.....	.....	.881	.....	.....
Fredonia, N. Y	1	1	14.7	15.71	.....	.....	2.25	.....	.....	13.46	.....	.....	.904	.....	.....
Averages, limits, and totals	2	2	17.0	18.27	.....	.....	2.46	.....	.....	15.81	.....	.....	.893	.....	.....
Herbert.															
Geneva, N. Y	1	1	18.0	19.13	.....	.....	2.59	.....	.....	16.54	.....	.....	1.200	.....	.....
Hexamer 24-20.															
Geneva, N. Y	1	1	19.4	20.10	.....	.....	3.74	.....	.....	16.36	.....	.....	1.245	.....	.....
Hicks.															
Geneva, N. Y	1	1	15.0	15.79	.....	.....	2.47	.....	.....	13.32	.....	.....	1.159	.....	.....
Illinois City.															
Geneva, N. Y	1	1	18.8	20.07	.....	.....	2.26	.....	.....	17.81	.....	.....	.998	.....	.....
Iona.															
Middle Bass, Ohio	1	1	(1)	23.17	.....	.....	2.39	.....	.....	20.78	.....	.....	.664	.....	.....
North Bass, Ohio	2	2	21.5	24.03	25.07	22.99	2.47	2.61	2.32	21.57	22.46	20.67	.904	1.155	0.653
Vermilion, Ohio	2	2	21.5	23.67	23.88	23.46	2.36	2.38	2.34	21.31	21.50	21.12	.928	.956	.900
Geneva, N. Y	1	1	19.6	21.25	.....	.....	2.83	.....	.....	18.42	.....	.....	1.426	.....	.....
Portland, N. Y	1	1	21.5	23.83	.....	.....	2.46	.....	.....	21.37	.....	.....	1.046	.....	.....
Pulteney, N. Y	1	1	21.4	23.41	.....	.....	2.51	.....	.....	20.90	.....	.....	1.234	.....	.....
Averages, limits, and totals	8	8	21.2	23.38	25.07	22.99	2.48	2.38	2.32	20.90	22.46	20.67	1.004	1.155	.653
Isabella.															
Kelleys Island, Ohio	1	1	19.1	21.04	.....	.....	2.26	.....	.....	18.78	.....	.....	.716	.....	.....
North Bass, Ohio	1	1	20.0	21.83	.....	.....	2.12	.....	.....	19.71	.....	.....	.836	.....	.....
Hammondsport, N. Y	1	1	20.0	21.88	.....	.....	2.11	.....	.....	19.77	.....	.....	.773	.....	.....
Pulteney, N. Y	1	1	16.7	17.88	.....	.....	2.44	.....	.....	15.44	.....	.....	1.016	.....	.....
Urbana, N. Y	4	4	16.6	17.79	19.58	16.31	2.50	2.74	2.31	15.29	16.97	13.57	1.028	1.076	.956
Wayne, N. Y	1	1	17.1	18.56	.....	.....	2.67	.....	.....	15.89	.....	.....	1.163	.....	.....
Averages, limits, and totals	9	9	17.6	19.15	19.58	16.31	2.40	2.74	2.31	16.75	16.97	13.57	.957	1.076	.956

[1] Brix not read.

TABLE III.—*Average, maximum, and minimum data for 1910, arranged by varieties and localities*—Continued.

Variety and locality.	Number of samples.	Number of growers.	Brix.	Total solids.			Sugar-free solids.			Sugar as invert.			Acid as tartaric.		
				Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.
ISABELLA SEEDLING.															
Geneva, N. Y	1	1	15.6	16.47	.....	.....	2.84	.....	.....	13.63	.....	.....	1.174	.....	.....
IVES.															
Ceylon, Ohio	3	3	18.4	19.34	20.55	18.45	2.43	2.52	2.32	16.92	18.23	16.03	0.678	0.844	0.559
Danbury, Ohio	1	1	15.4	16.62	.....	.....	2.71	.....	.....	13.91	.....	.....	.608	.....	.....
Kelleys Island, Ohio	1	1	17.5	18.63	.....	.....	2.09	.....	.....	16.54	.....	.....	.555	.....	.....
Middle Bass, Ohio	9	8	16.4	17.41	19.31	14.93	2.47	2.73	2.23	16.92	16.68	12.37	.823	.960	.713
Venice, Ohio	2	2	[1] 18.3	18.66	19.81	17.51	2.77	2.95	2.59	15.89	16.86	14.92	.733	.746	.720
Vermilion, Ohio	2	2	20.1	21.36	21.44	21.28	2.45	2.55	2.35	18.91	18.93	18.89	.655	.683	.626
Bluff Point, N. Y	1	1	19.6	21.33	.....	.....	2.48	.....	.....	18.85	.....	.....	.641	.....	.....
Hammondsport, N. Y	1	1	17.9	19.42	.....	.....	2.52	.....	.....	16.90	.....	.....	.836	.....	.....
North East, Pa.: South Shore Wine Co	2	1	16.3	17.20	18.22	16.18	3.02	3.08	2.96	14.18	15.14	13.22	1.063	1.151	.975
Averages, limits, and totals	22	20	17.3	18.42	21.44	14.93	2.54	3.08	2.23	15.88	18.23	12.37	.772	1.151	.559
JANESVILLE.															
Geneva, N. Y	1	1	16.6	17.48	.....	.....	3.53	.....	.....	13.95	.....	.....	1.462	.....	.....
LINDLEY.															
Fredonia, N. Y	1	1	15.5	16.13	.....	.....	2.69	.....	.....	13.44	.....	.....	1.155	.....	.....
Geneva, N. Y	1	1	18.8	20.26	.....	.....	2.49	.....	.....	17.77	.....	.....	.953	.....	.....
Averages, limits, and totals	2	2	17.2	18.20	.....	.....	2.59	.....	.....	15.61	.....	.....	1.054	.....	.....
LUCILE.															
Fredonia, N. Y	1	1	15.6	16.15	.....	.....	2.70	.....	.....	13.45	.....	.....	.915	.....	.....
Geneva, N. Y	1	1	(2)	17.59	.....	.....	2.12	.....	.....	15.47	.....	.....	.814	.....	.....
Averages, limits, and totals	2	2	[1] 15.6	16.87	.....	.....	2.41	.....	.....	14.46	.....	.....	.865	.....	.....
MARION.															
Geneva, N. Y	2	1	19.0	21.07	21.15	20.99	3.77	3.83	3.70	17.31	17.32	17.29	1.566	1.610	1.522
MASSASOIT.															
Geneva, N. Y	1	1	16.3	17.09	.....	.....	1.88	.....	.....	15.21	.....	.....	.754	.....	.....
MILLS.															
Portland, N. Y	2	1	20.0	21.99	22.07	21.91	2.14	2.15	2.13	19.85	19.94	19.76	.741	.754	.728
MISSOURI RIESLING.															
Kelleys Island, Ohio	1	1	19.4	20.78	.....	.....	2.42	.....	.....	18.36	.....	.....	.941	.....	.....
MONTEFIORE.															
Kelleys Island, Ohio	1	1	(2)	23.65	.....	.....	2.29	.....	.....	21.36	.....	.....	.784	.....	.....
MOORE DIAMOND.															
Fredonia, N. Y	1	1	18.0	19.52	.....	.....	3.06	.....	.....	16.46	.....	.....	1.121	.....	.....
MOORE EARLY.															
Fredonia, N. Y	1	1	15.1	15.87	.....	.....	.....	.....	.....	9.04	.....	.....	.825	.....	.....

[1] One sample.

[2] Brix not read.

TABLE III.—*Average, maximum, and minimum data for 1910, arranged by varieties and localities*—Continued.

Variety and locality.	Number of samples.	Number of growers.	Brix.	Total solids.			Sugar-free solids.			Sugar as invert.			Acid as tartaric.		
				Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.
MOYER.															
Fredonia, N. Y	1	1	18.5	20.00	....	....	2.50	....	....	17.50	....	....	0.780	....	....
Geneva, N. Y	1	1	19.1	20.12	....	....	2.40	....	....	17.72	....	....	.641	....	....
Averages, limits, and totals	2	2	18.8	20.06	....	....	2.45	....	....	17.61	....	....	.711	....	....
R. W. MUNSON.															
Geneva, N. Y	1	1	14.7	15.48	....	....	2.91	....	....	12.57	....	....	1.490	....	....
NECTAR.															
Geneva, N. Y	1	1	18.3	19.79	....	....	2.53	....	....	17.26	....	....	1.256	....	....
NIAGARA.															
North Bass, Ohio	2	1	19.2	21.08	21.54	20.62	2.17	2.30	2.03	18.92	19.24	18.59	.448	0.450	0.446
Benton, N. Y	1	1	15.6	16.91	....	....	2.21	....	....	14.70	....	....	.754	....	....
Fredonia, N. Y	1	1	15.3	16.02	....	....	3.13	....	....	12.89	....	....	.968	....	....
Geneva, N. Y	1	1	12.9	13.42	....	....	2.06	....	....	11.36	....	....	.773	....	....
Penn Yan, N. Y	1	1	12.6	14.38	....	....	2.71	....	....	11.67	....	....	.840	....	....
North East, Pa.: South Shore Wine Co.	1	1	17.8	19.08	....	....	2.37	....	....	16.71	....	....	.806	....	....
Lawton, Mich	1	1	17.0	18.03	....	....	2.13	....	....	15.90	....	....	.791	....	....
Averages, limits, and totals	8	7	16.2	17.50	21.54	20.62	2.37	2.30	2.03	15.13	19.24	18.59	.728	....	....
NOAH.															
Geneva, N. Y	1	1	16.5	17.33	....	....	3.08	....	....	14.25	....	....	1.632	....	....
NORFOLK.															
Geneva, N. Y	1	1	17.4	18.58	....	....	2.48	....	....	16.10	....	....	1.144	....	....
NORTON.															
Kelleys Island, Ohio	1	1	22.8	24.27	....	....	3.53	....	....	20.74	....	....	1.856	....	....
North Bass, Ohio	1	1	23.9	26.54	....	....	3.65	....	....	22.89	....	....	1.522	....	....
Venice, Ohio	2	2	(1)	24.16	24.20	24.12	3.54	3.59	3.49	20.62	20.63	20.61	1.787	1.942	1.632
Vermilion, Ohio	1	1	23.5	26.01	....	....	3.85	....	....	22.16	....	....	1.462	....	....
Averages, limits, and totals	5	5	23.4	25.43	24.20	24.12	3.62	3.59	3.49	21.41	20.63	20.61	1.683	1.942	1.632
OLITA.															
Geneva, N. Y	1	1	17.0	18.16	....	....	2.19	....	....	15.97	....	....	.908	....	....
PIERCE.															
Geneva, N. Y	1	1	14.0	14.87	....	....	2.45	....	....	12.42	....	....	1.253	....	....
POCKLINGTON.															
North Bass, Ohio	1	1	17.8	19.16	....	....	1.87	....	....	17.29	....	....	.596	....	....
Venice, Ohio	1	1	18.0	19.31	....	....	2.53	....	....	16.78	....	....	1.095	....	....
Averages, limits, and totals	2	2	17.9	19.24	....	....	2.20	....	....	17.04	....	....	.846	....	....
REGAL.															
Portland, N. Y	1	1	16.8	18.14	....	....	2.03	....	....	16.11	....	....	.836	....	....
REQUA.															
Geneva, N. Y	2	1	17.9	19.23	20.70	17.75	2.27	2.36	2.18	16.75	18.52	14.97	.844	.960	.728
ROGERS NO. 24.															
Geneva, N. Y	1	1	15.0	15.66	....	....	2.47	....	....	13.19	....	....	1.238	....	....

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[1] Brix not read.

TABLE III.—*Average, maximum, and minimum data for 1910, arranged by varieties and localities*—Continued.

Variety and locality.	Number of samples.	Number of growers.	Brix.	Total solids.			Sugar-free solids.			Sugar as invert.			Acid as tartaric.		
				Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.
ROGERS NO. 32.															
Geneva, N. Y	1	1	16.9	17.85	.....	.....	2.42	.....	.....	15.43	.....	.....	1.181	.....	.....
RUPERT.															
Geneva, N. Y	1	1	16.1	16.83	.....	.....	3.55	.....	.....	13.28	.....	.....	1.756	.....	.....
SALEM.															
North Bass, Ohio	1	1	20.5	22.49	.....	.....	1.58	.....	.....	20.91	.....	.....	.521	.....	.....
Bluff Point, N. Y	1	1	18.9	20.60	.....	.....	2.78	.....	.....	17.82	.....	.....	.938	.....	.....
Pulteney, N. Y	1	1	22.2	24.41	.....	.....	2.63	.....	.....	21.78	.....	.....	1.024	.....	.....
Averages, limits, and totals	3	3	20.5	22.50	.....	.....	2.33	.....	.....	20.17	.....	.....	.828	.....	.....
ST. LOUIS.															
Geneva, N. Y	1	1	15.2	15.76	.....	.....	2.45	.....	.....	13.31	.....	.....	1.140	.....	.....
SCHRAIDTES.															
Middle Bass, Ohio	1	1	19.7	21.15	.....	.....	3.86	.....	.....	17.29	.....	.....	1.632	.....	.....
North Bass, Ohio	1	1	20.9	23.20	.....	.....	3.93	.....	.....	19.27	.....	.....	1.556	.....	.....
Averages, limits, and totals	2	2	20.3	22.18	.....	.....	3.90	.....	.....	18.28	.....	.....	1.594	.....	.....
TELEGRAPH.															
Fredonia, N. Y	1	1	16.3	17.46	.....	.....	3.56	.....	.....	13.90	.....	.....	1.256	.....	.....
TRIUMPH.															
Geneva, N. Y	1	1	(1)	16.88	.....	.....	2.79	.....	.....	14.09	.....	.....	1.354	.....	.....
UNKNOWN.															
Kelleys Island, Ohio	1	1	23.8	26.43	.....	.....	2.04	.....	.....	24.39	.....	.....	.626	.....	.....
Middle Bass, Ohio	3	2	[2] 18.9	21.23	22.54	20.05	2.49	2.63	2.30	18.74	19.91	17.50	.677	0.743	0.623
Venice, Ohio	3	2	23.6	26.02	28.49	24.38	2.39	2.77	2.01	23.84	26.11	22.37	.657	.848	.439
Vermilion, Ohio	3	1	20.3	22.66	22.72	22.59	2.68	2.72	2.64	18.23	20.00	14.75	1.075	1.144	1.005
Fredonia, N. Y	1	1	16.1	17.62	.....	.....	3.65	.....	.....	13.97	.....	.....	1.354	.....	.....
Averages, limits, and totals	11	7	21.0	23.11	28.49	20.05	2.58	2.77	2.01	20.55	26.11	14.75	.813	1.144	.439
TRAGEDY.															
Geneva, N. Y	1	1	(1)	18.82	.....	.....	2.17	.....	.....	16.65	.....	.....	.776	.....	.....
VERGENNES.															
Geneva, N. Y	1	1	18.1	19.50	.....	.....	2.72	.....	.....	16.78	.....	.....	1.163	.....	.....
Portland, N. Y	2	1	18.4	19.92	20.26	19.58	2.31	2.50	2.11	17.62	18.15	17.08	1.041	1.129	.953
Averages, limits, and totals	3	2	18.3	19.78	.....	.....	2.44	.....	.....	17.34	.....	.....	1.082	.....	.....
WAPENUKA.															
Geneva, N. Y	1	1	12.9	13.47	.....	.....	4.05	.....	.....	9.42	.....	.....	1.084	.....	.....
WILDER.															
Fredonia, N. Y	1	1	15.6	16.49	.....	.....	2.38	.....	.....	14.11	.....	.....	1.189	.....	.....
WORDEN.															
Venice, Ohio	10	2	[3] 18.3	19.33	22.51	15.08	2.34	2.95	2.00	16.99	20.28	12.13	.876	1.283	.634
Fredonia, N. Y	1	1	12.8	13.49	.....	.....	2.54	.....	.....	10.95	.....	.....	1.372	.....	.....
Geneva, N. Y	3	1	13.8	14.68	14.80	14.56	2.45	2.53	2.36	12.56	13.21	12.03	1.271	1.312	1.211
Penn Yan, N. Y	1	1	13.1	14.20	.....	.....	2.27	.....	.....	11.93	.....	.....	1.020	.....	.....
Averages, limits, and totals	15	5	14.3	17.88	.....	.....	2.36	.....	.....	16.46	.....	.....	.998	.....	.....

[1] Brix not read. [2] Two samples. [3] One sample.

TABLE III.—*Average, maximum, and minimum data for 1910, arranged by varieties and localities*—Continued.

Variety and locality.	Number of samples.	Number of growers.	Brix.	Total solids.			Sugar-free solids.			Sugar as invert.			Acid as tartaric.		
				Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.
WYOMING RED.															
Fredonia, N. Y.	1	1	15.9	16.78	.....	.....	2.95	.....	.....	13.83	.....	.....	0.855	.....	.....
Geneva, N. Y.	1	1	14.1	14.85	.....	.....	2.09	.....	.....	12.76	.....	.....	.754	.....	.....
Averages, limits, and totals	2	2	15.0	15.82	.....	.....	2.52	.....	.....	13.29	.....	.....	.805	.....	.....
YONKEN HONEY DEW.															
Geneva, N. Y.	1	1	17.9	19.10	.....	.....	2.76	.....	.....	16.34	.....	.....	.956	.....	.....
No. 3197	1	1	19.6	22.36	.....	.....	2.26	.....	.....	20.10	.....	.....	.758	.....	.....
No. 4069	1	1	15.7	16.68	.....	.....	1.72	.....	.....	14.96	.....	.....	.604	.....	.....
No. 2698	1	1	15.2	15.87	.....	.....	1.56	.....	.....	14.31	.....	.....	.510	.....	.....
No. 98	1	1	19.3	20.83	.....	.....	1.79	.....	.....	19.04	.....	.....	.544	.....	.....
No. 116	1	1	15.8	16.68	.....	.....	1.83	.....	.....	14.85	.....	.....	.529	.....	.....
No. 3345	1	1	13.6	14.09	.....	.....	1.87	.....	.....	12.22	.....	.....	.731	.....	.....
No. 2717	1	1	17.0	18.08	.....	.....	1.89	.....	.....	16.19	.....	.....	.645	.....	.....
No. 2652	1	1	19.9	21.31	.....	.....	2.06	.....	.....	19.25	.....	.....	.645	.....	.....

TABULATION OF ANALYTICAL DATA ON SAMPLES GROWN AND ANALYZED AT CHARLOTTESVILLE, VA.

TABLE IV.—*Average, maximum, and minimum data, 1908–1910, arranged by varieties.* (*Analyses by Hartmann and Eoff.*)

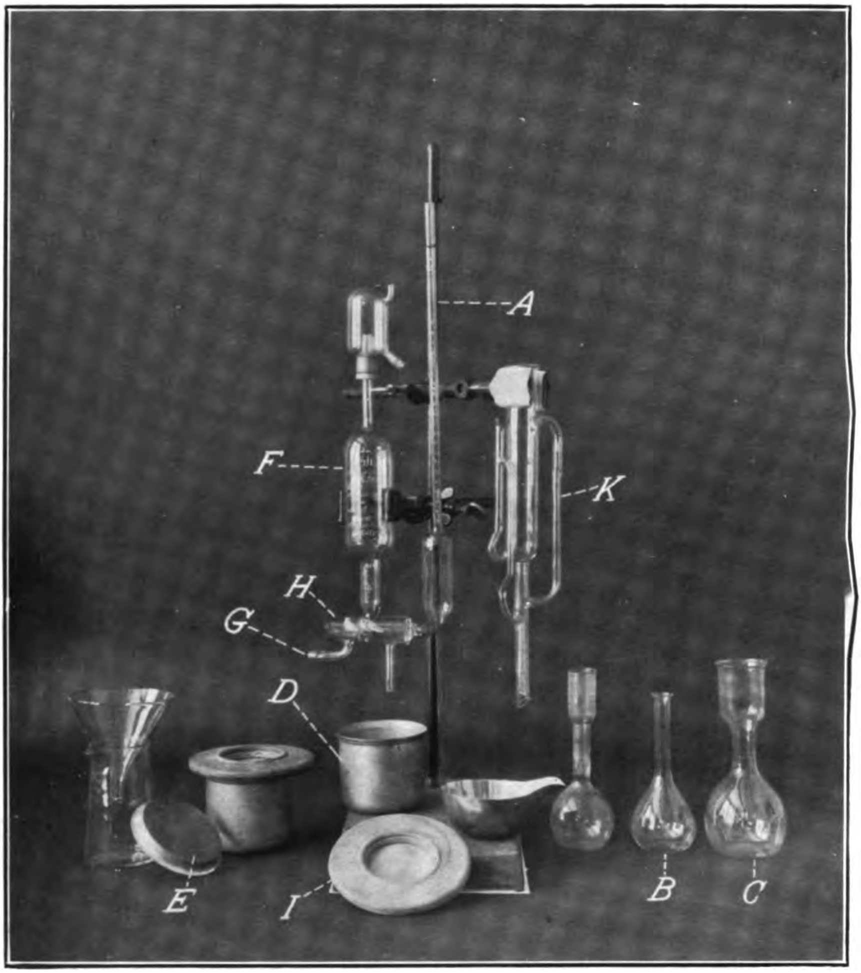
[Results expressed in grams per 100 cc of juice.]

Year.	Variety.	Number of samples.	Number of growers.	Specific gravity.	Total solids.			Sugar-free solids.			Total sugar as invert.			Acid as tartaric.		
					Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.
1908	Norton	9	7	1.1009	26.26	27.88	24.46	3.64	4.50	2.97	22.63	24.73	19.99	1.088	1.290	0.975
1909	Concord	14	5	1.0731	18.94	20.49	16.99	2.99	3.54	2.59	16.01	17.50	14.08	.744	.840	.645
1909	Cynthiana	1	1	1.0918	23.91	.....	.....	4.16	.....	.....	19.75	.....	.....	.986	.....	.....
1909	Delaware [1]	2	.....	1.0969	25.30	26.43	24.17	2.86	3.08	2.63	22.45	23.80	21.09	.702	.784	.619
1909	Elvira	1	1	1.0698	18.11	.....	.....	2.76	.....	.....	15.35	.....	.....	.735	.....	.....
1909	Ives	12	7	1.0702	18.26	22.09	15.92	3.15	3.54	2.79	15.11	18.60	13.00	.753	.866	.653
1909	Noah	2	1	1.0876	22.80	22.88	22.72	2.75	2.75	2.74	20.06	20.13	19.98	.957	.990	.923
1909	Norton	11	9	1.0957	24.93	25.88	23.49	3.56	3.98	2.92	21.35	22.45	20.00	.925	1.001	.776
1909	Riesling	1	1	1.0647	16.99	.....	.....	2.69	.....	.....	14.30	.....	.....	.851	.....	.....
1910	Concord [2]	12	5	1.0747	19.44	21.65	17.04	3.11	3.61	2.60	16.33	18.15	14.07	.823	.904	.724
1910	Cynthiana	1	1	1.0962	25.04	.....	.....	3.92	.....	.....	21.10	.....	.....	1.080	.....	.....
1910	Delaware [1]	1	.....	1.0888	23.12	.....	.....	2.12	.....	.....	21.00	.....	.....	.551	.....	.....
1910	Ives	15	9	1.0714	18.60	20.23	15.00	3.09	4.74	2.31	15.38	16.85	12.69	.714	.908	.611
1910	Norton	23	10	1.1003	26.23	28.54	24.17	3.88	4.63	3.37	22.44	25.04	20.10	.957	1.328	.750

[1] Mixed crops.

[2] Three samples from Crozet, Va.

O



APPARATUS FOR BEET ANALYSIS.

A, Sucrose pipette. *B*, Sugar flask. *C*, Flask for cold or hot water digestion. *D*, *E*, *I*, Metal cups and covers for the Sachs Le Docte method. *F*, Automatic pipette for the Sachs Le Docte method. *K*, Modified Soxhlet extractor for alcohol extraction method.

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ANALYSES OF SUGAR BEETS, 1905 TO 1910,

TOGETHER WITH

METHODS OF SUGAR DETERMINATION.

BY

A. HUGH BRYAN,
Chief, Sugar Laboratory.

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LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Chemistry,
Washington, D. C., June 14, 1911.

Sir: I have the honor to submit for your approval a report prepared in the sugar laboratory of this bureau on the analyses of sugar beets made during the years 1905 to 1910, inclusive, together with methods of determining the percentage of sugar in the beet. This compilation, representing, as it does, beets from many different localities, will be useful in answering the many inquiries received as to the quality of beets grown in the various sections of the country and the best methods for determining their sugar content. I recommend, therefore, that the manuscript be published as Bulletin No. 146 of the Bureau of Chemistry.

Respectfully,
H. W. Wiley,
Chief of Bureau.

Hon. James Wilson,
Secretary of Agriculture.

CONTENTS.

Page.
Introduction..... 5
Factors to be considered in interpreting analytical results..... 6
Methods of sampling..... 8
  Sampling from the field..... 9
  Sampling from pile, wagon, or car..... 9
  Laboratory sampling and pulping..... 10
  Sampling and testing seed beets..... 12
Methods for determining sugar..... 14
  Indirect method..... 14
    Errors of the method..... 15
    Hydraulic press..... 17
  Direct methods..... 17
    Water methods..... 17
      Cold-water digestion method of Pellet..... 17
      Cold-water digestion method of Sachs Le Docte..... 18
      Hot-water digestion method..... 18
      Hot-water digestion method of Sachs Le Docte..... 19
      Herzfeld's modification of the Sachs Le Docte method..... 19
    Alcohol methods..... 20
      Cold alcohol digestion method..... 20
      Hot alcohol digestion method..... 21
      Alcohol extraction method..... 21
  Discussion of methods..... 22
Detailed analytical data, 1905 to 1910..... 23
Yearly average analytical data by States, from 1884 to 1900 and from 1905 to 1910..... 36
Bibliography of chemical literature on methods of analyzing beets (1839 to 1906, inclusive)..... 40

ILLUSTRATIONS.

PLATES.

Page.
Apparatus for beet analysis.....Frontispiece.
PLATE I. Hydraulic press..... 16

TEXT FIGURES.

FIG. 1. Horizontal sectioning of beets..... 10
2. Vertical sectioning of beets..... 11
3. Correct position of boring rasp in beet..... 13
4. Boring rasp..... 13
5. Herzfeld's metallic beaker..... 20

ANALYSES OF SUGAR BEETS, 1905 TO 1910,

TOGETHER WITH

METHODS OF SUGAR DETERMINATION.

INTRODUCTION.

Many requests are received at this department for reports on analyses of beets grown in different localities and also for information as to the fitness of certain sections for growing beets. Many inquiries are also received as to the best methods for the analysis of beets. This report, including a résumé of the analyses made of beets grown in various sections of this country throughout a period of six years, and also a statement of the methods used for beet analysis, with a discussion of their comparative accuracy, has been compiled to meet this demand.

As early as 1862 [1] the Department of Agriculture became interested in sugar-beet work, and for the past 30 years this bureau has been engaged in the analysis of sugar beets and in making investigations for the improvement of this industry. The following bulletins have been published giving the results of this work: No. 3*, The Northern Sugar Industry, 1883; No. 5*, The Sugar Industry of the United States, 1885; No. 27*, The Sugar-Beet Industry, 1890; Nos. 30*, 33*, 36*, 39*, and 52*, devoted to experiments with sugar beets in the years 1890, 1891, 1892, 1893, and 1897; Nos. 64, 74, 78, 95, and 96, devoted to a five years' study of the influence of environment upon the composition of the sugar beet, undertaken in 1900 to 1904, inclusive.

The bulletins marked with an asterisk (*) are out of print and are not available for distribution; the others may be had on application. Also, as a part of Progress of the Beet Sugar Industry of the United States, published yearly as a special report of the Secretary of Agriculture, there has been issued in the years 1897, 1898, 1899, and 1900 a report covering the analytical figures obtained in the analyses of samples of beets for these years. Since 1900 there has been no publication of results of analyses of beets made in the Bureau of Chemistry other than those published in the study of the influence of environment on the composition of the beet. Many samples have been analyzed since that time.

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[1] U. S. Dept. Agr., Bureau of Chemistry Bul. 52, p. 12.

During the years 1901–1904 many samples of beets were analyzed for local sugar projects, but their results are not included. The results from 1905 on to 1910 have been tabulated by States and counties. The location of the county in the State is noted by the usual sign, namely: □ center of State; □- east of center of State; ⟋□ southwest of center of State, etc. The figures given are for the average weight of beets (expressed in ounces), per cent of sugar in juice, and purity of the juice (the per cent of sugar in the solids of the juice). The condition of the sample as received is also stated. In this table no averages for States or counties are given, as so many factors enter into the results that conclusions based on such data might be misinterpreted. In fact, one can only form a comparative opinion as to the fitness of a certain region for beet growing from these analyses. The reasons for this may be briefly stated and serve also to illustrate the need for very careful work before passing final judgment on the possibilities of any section for beet culture.

FACTORS TO BE CONSIDERED IN INTERPRETING ANALYTICAL RESULTS.

A sugar beet is a plant that is greatly influenced by environment, cultivation, etc. Beets from the same seed may be grown in the same soil and under the same climatic conditions and the sugar contents at maturity be very different, owing to different methods of cultivation. A farmer who has grown beets a number of years in succession may raise a better crop than one in the same locality who has had no experience. Many of the samples herein reported came from farmers who had never raised beets before; some of the crops were no doubt raised on land entirely unsuitable for beet culture, and the results from the latter experiments would be of value as a test only if the soil of the plat were representative of this particular section. The large number of persons applying for beet seeds during a year made it impossible to keep a detailed record of the varying cultural and climatic conditions, although instructions were sent in all cases regarding the selection of the plat for the work and cultivation methods.

Correct sampling methods are also important to insure comparable results. Many people believe that the more prolific the growth of a beet the higher will be the percentage of sugar, and so select large beets from a patch for testing. This is not true, however, except in rare cases. Again, the sugar content varies with the degree of ripeness. As a beet matures the percentage of sugar increases; so selection with regard to this point is important. Two persons going through a field for sampling may draw samples that will vary as much as 3 or 4 per cent in sugar content. But if one is familiar with the work, the results from 20 or even fewer beets may fairly represent the field at that particular stage of growth. As no attention was paid to the method of sampling in over 90 per cent of the cases here reported, the results, as a basis for judging of the suitability for a given area for beet cultivation, are apt to be misleading.

Another factor which may affect the amount of sugar found is the condition of the beets when received for analysis. The healthy beet when taken from the ground is crisp, but on exposure to air and heat it soon loses moisture and becomes wilted; the next step is rotting. In losing water, the percentage of sugar present will, of course, be increased, but this increase is seldom in the same ratio as the loss in weight. Experiments conducted in 1891[1] at the Schuyler station in Nebraska show what may happen under these conditions. Beets were dug on October 3, carefully cleaned, and the leafy tops removed. They were then placed in the sun and reweighed at the end of each 24 hours. The daily temperature was 68° F. and the mean maximum 90° F. A heavy wind was blowing most of the time. Beginning with 152 pounds of beets, the loss in weight after one day was 13.2 per cent, after two days 23.8 per cent, after three days 32.4 per cent, and after four days 37.5 per cent. A sample drawn from the fresh beets showed 15.1 per cent of sugar, while at the end of the fourth day a sample drawn showed 17.1 per cent, an increase of only 2 per cent of sugar. Calculating the original sugar content of 15.1 per cent for the loss in weight, the beets should contain at the end of the four days 24.2 per cent, showing a loss of 7.1 per cent in sugar in the beet. This is a remarkable loss, but the experiment was carried on under severe weather conditions, great heat and wind.

A second trial was made in which clean beets were divided into three portions of 25 pounds each. One portion was left in the field, another was kept in the air but under a shed where the direct rays of the sun did not come in contact with the beets, while the third portion was analyzed. At the end of three days there was a 20 per cent loss in weight for the shed beets and a 22 per cent decrease for the field beets. The sugar content of the fresh beets was 16.2 per cent, of the shed beets 19.6 per cent, and of the field beets 18.3 per cent. Figuring the loss in weight as moisture, the sugar percentage of the shed beets should be 20.2 per cent at the end of the three days and of the field beets 20.7 per cent. The sugar losses here noted are not so large as in the previous experiment, being only 0.6 per cent when beets were kept away from the direct rays of the sun, but 2.4 per cent when they were placed in the sun.

Other experiments that have been tried indicate that after harvesting sun and heat work great detriment to the sugar content. An interesting experiment, showing that under certain conditions the sugar content does increase in proportion to the loss in weight, was tried at the same station. Twenty beets of about the same size and of the same degree of ripeness were selected. Ten were analyzed immediately and the other 10 were wrapped in oiled paper and sent to Washington for examination. The results are given in the following table:

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[1] U. S. Dept. Agr., Bureau of Chemistry, Bul. 36, p. 62.

Comparative analyses before and after shipment.

Time of analysis.	Loss in weight.	Per cent of sugar in beets.	Grams of sugar in beets.
	Per cent.		
Fresh		14.7	47.9
After shipment to Washington	12	16.6	47.9

There was a loss in weight during shipping of 12 per cent, but an increase of 1.9 per cent in the beet is noted, and the actual amount of sugar in the beet remains unchanged.

In the case of the samples herein reported the directions for shipping beets to Washington for analysis were to wrap each beet separately in oil paper, this paper being sent the grower, together with the shipping tag. In many cases the beets were fresh when received, but in a large percentage of instances they had evidently dried out before wrapping them for sending. It is important in using the analytical data contained in the tables to remember that these factors may have influenced the results.

For commercial purposes a beet of over 12 per cent sugar content, 12.5 to 13.5 per cent of sugar in the juice, and at least 80 per cent purity, and which weighs over 1 pound but under 4 or 5 pounds, is considered the most desirable. The contracts of most sugar companies with the farmers are based on these figures, but at times deviations from them are accepted. With these facts in mind one may form an opinion of the manufacturing value of the beets reported in the tabulated data (see p. 23).

METHODS OF SAMPLING.

The accuracy of the determination of sugar in the beet depends largely upon mechanical processes and accurate results are difficult to obtain unless a stated procedure is strictly followed, thus eliminating many chances of error. As with many other procedures, the statement of methods for the analysis of sugar beets is not sufficiently detailed and its limitations are not pointed out, so that an inexperienced worker may obtain accurate results. For example, all methods for beet analysis presuppose a finely divided pulp. With some methods the pulp must be much finer than with others. This is especially true of "instantaneous" methods or those obtaining the sugar by digestion in the cold. The sampling and subsampling of the beets are important details, for unless the sample is representative the results obtained are of little value.

SAMPLING FROM THE FIELD.

The beet chemist is often called upon to take a sample from the field. This sample should accurately represent the whole crop to be of any value as an index of the condition of the field. There are many methods of accomplishing this, some of a mathematical nature. The method to be employed depends somewhat upon the size of the field and the purpose for which the results are to be used. If the plat is small and it is the purpose to sample often, then a smaller sample may be used, but when the fields are extensive a larger sample should be drawn.

As an example of a mathematical method of sampling, the following is given: Look over the plat, noting the stand, and remove all beets that are not up to the average condition of the field. Go through again and dig every fourth to tenth beet (according to size of plat) for the analytical sample; every twentieth beet should be taken if the plat is very large. From 20 to 100 beets should constitute a sample. Another method is to remove all the beets in one row or a measured portion of a row and use these as the analytical sample. Care must be exercised that the row selected is representative.

To ascertain for factory purposes whether or not the crop is ripe and ready for harvest, the general practice is to walk across the field from one corner to the opposite one, noting the stand and the general condition of the beets as indicated by their leaves, general appearance, etc.; then on returning to dig from 5 to 20 beets at random, selecting those in average condition. Here the judgment of the sampler is wholly relied upon, but with experience and a knowledge of the variety under cultivation, a representative sample should be obtained.

Whatever method is used, it is essential in digging and cleaning the beets not to injure the skin and not to break the roots too short. The topping should consist only in removing the leaves, the crown being left in place for the chemist to remove; this prevents drying of the sample, etc.

SAMPLING FROM PILE, WAGON, OR CAR.

In sampling from a pile instead of from a field the judgment of the sampler again must be relied upon. A good procedure is to examine the pile of beets, noting the general shape and average size, and then to select from 10 to 50 beets for analysis, depending on the size of the pile. A proportionate number of large, small, and irregular-shaped beets should be used as they occur in the pile.

In drawing samples from a wagonload or carload of beets, it is customary to adopt one of the following procedures: When a dumping wagon is used, a bushel basket is placed on the bin in the path of the falling beets and withdrawn after the load is dumped. The beets in the basket constitute the analytical sample. If an ordinary wagon is used a bushel basket full of the beets is forked out during the unloading. In case the sample is taken directly from the cars, three or more samples should be selected from different portions of the car as it is being unloaded in the same manner as from the ordinary wagon.

LABORATORY SAMPLING AND PULPING.

The sampling of beets in the laboratory is a very important matter, as for the final analysis only a very small portion is used. The samples offered for analysis are composed of from 10 to 100 beets, and in an ordinary laboratory it is practically impossible to pulp all of the larger sample; therefore, one must resort to removing a section from each beet and pulping this. The removal of the right section is a difficult matter, since the sugar is not evenly distributed throughout the beet. Many persons have studied the localization of the sugar content of the beet. Prominent among them are Violette, Wiesner, De Vries, and Marek.[1] The latter went into the subject quite thoroughly in 1882. Proskowetz a number of years later worked out the localization of the sugar content in the beet as follows: By dividing a beet into four or five equal horizontal sections, as is shown in figure 1, he obtained the following percentages of sugar in the various portions:

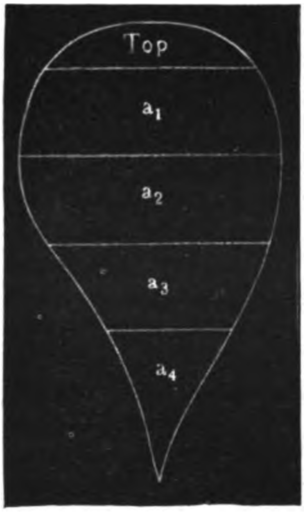

FIG. 1.—Horizontal sectioning of beets.

Percentages of sugar in horizontal sections of the beet.

Part of beet.	Sample 1.	Sample 2.	Sample 3.
In top....	12.30	14.08	17.52
A_1........	12.62	14.36	17.82
A_2........	12.57	14.42	17.68
A_3........	11.95	14.50	17.26
A_4........	(1)	14.20	(1)

(1) Only four sections made.

From this it is shown that the bulged section (A_1) has the highest percentage of sugar. Other observers by dividing the beet into more and smaller sections obtained the same result, namely, that the highest sugar content is located in the region of the point of gravity of the beet mass and diminishes on both sides toward the top and the tip. Dividing the beet as is shown in figure 2—that is, cutting it into circular portions from the center to the skin—the following results were obtained:

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[1] Within the last few months Floderer and Herke have reviewed the previous work on this subject and have also added a large amount of careful experimental work. They come to practically the same conclusions as are here given. Osterr.-Ung. Zts. Zuckerind. Land., 1911, 40: 385.

Percentage of sugar in different vertical sections of the beet.

Section of beet.	Per cent sugar.
B_1	13.99
B_2	14.12
B_3	14.13
B_4	12.98

From these data it is seen that the zone next to the outside of the beet contains the highest sugar per cent, the next inside zone being nearly as high, while the outside and the central zones are lower. If the beet were cut into more zones, it would be found that the highest sugar occurred in a zone about three-fourths of the way from center to the outside.

Plant physiology shows that some cells contain much more sugar than others. If the beet is perfect in form a section taken throughout its complete length and passing to the center of the beet would be representative. If the beet is not of even form an error is apt to creep in, as it has been found that the per cent of sugar is higher in the portion of the beet that is depressed than in the well-expanded portions. If the weights of the sections removed in sampling could be so regulated that they bore an exact ratio to the whole weight of the beet, and this were constant for all, then these sections would accurately represent the large sample. Unfortunately this condition can only be approached, as it is possible to cut a beet in half or even in quarters, but beyond that it is difficult to subdivide by hand. The hand method is the one usually employed, however, in sampling beets, although machines in the form of cone-shaped rasps, which remove a section from the beets, are manufactured. The rasp is circular in form, thicker at the center and tapering to the edge. By running a beet over this fast-revolving rasp, the section is removed in the form of a fine pulp, and may be caught in a basket or box placed below it. When using from 20 to 50 beets the sample of ground pulp is quite large, and for accurate work it must be rapidly mixed and quartered, drawing one or more samples for analysis. In mixing it is necessary

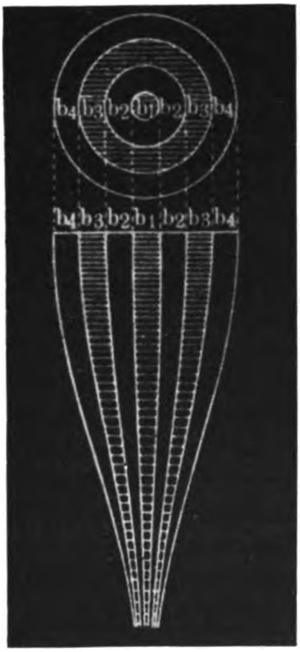

FIG. 2.—Vertical sectioning of beets.

to guard against evaporation and also squeezing of the pulp; evaporation causes, of course, a higher sugar content than is naturally present, while squeezing may result in either a higher or lower sugar content, depending on the sample drawn. With quick mixing, however, little loss occurs.

In case the section has been removed by hand, it must be reduced to a pulp. There are many machines made for this purpose; in general, any meat chopper which yields a finely divided product may be used, but some forms are preferable to others. An evenly cut or shredded pulp should be obtained by grinding with little or no pressing. Machines that operate by feeding the beets by means of a screw to the cutting knife are very liable to express some of the juice, while if the screw is fitted with knives which do the cutting as well as the feeding the pressure on the pulp is lessened. Other machines have a revolving bowl with rotatory knives constantly turning, the two being driven by the same power. This yields a fine pulp with little pressing, but is hardly capable of receiving halves or even quarters of beets to be sliced. An ordinary horse-radish grater serves admirably for reducing sections of beets to a fine pulp. This grater has a rotating drum provided with nails extending from its surface about a quarter of an inch. An adjustable chute extends to the drum, so that the beets can be fed to it by hand. The nails may be replaced by embedding hacksaw blades in the drum and allowing the coarse teeth to stand out above the surface. In using such a grater care must be exercised not to feed too fast and to have the carrier for the beets just touch the points of the drum. In this way the beet is cut squarely off and no portion goes through uncut. It is highly important to feed beets into this machine in such a way that the grater comes in contact with the skin of the beet first, otherwise it is difficult to shred the skin, which is generally very tough. When one sample is finished all of the beet particles must be carefully brushed off of the drum and machine to avoid mixing the samples. This, however, is true with any machine. After shredding, the sample may be thoroughly mixed by hand.

SAMPLING AND TESTING SEED BEETS.

Beets that are to be used for seed production are tested for sugar content, and it is essential to obtain a sample from them without injuring their productiveness. This is usually accomplished by boring a hole through the beet in some such way as is shown in figure 3, and collecting the borings for analysis. It is highly important to control the direction of the passage of this rasp through the beet. As has been shown, the sugar is not equally distributed throughout the beet. It behooves one, therefore, to cut through as many zones as possible and the same cut should be made in all cases if the results are to be comparable. Much work has been done to determine the point of sampling and it has been shown that the rasp should enter near the edge of the leaf scar and take a direction at an angle of 45° to the main axis through the beet. This can be accomplished by having the beet placed on a moving board tilted at the proper angle and pressed against the rasp, or the rasp may be pressed against the beet. Rasps are manufactured which remove this core whole, or collect the cuttings in the shaft of the drill (fig. 4). The sample obtained this way seldom amounts to 26 grams and it is difficult to press this quantity and obtain sufficient juice to determine the dry substance and also the sugar. The per cent of sugar is determined direct by one of the instantaneous methods or by the hot-water digestion method.[1] Ten grams of the beet pulp may be digested, made up to 38.7 cc; and polarized in a 200 mm tube; the reading will be the per cent of sugar. Or 6.48 grams of the cuttings may be digested, made up to 50 cc, and a reading made in a 400 mm tube. Again, the beet cuttings in the rasp can be placed in a tared dish and the whole weighed. The pulp is then washed into a 100 cc flask if the sample amounts to about 13 grams, or into a 200 cc flask if it is nearly 26 grams, and a water digestion is made in the usual way. The per cent of sugar obtained, multiplied by the relation of the weight of sample used to 26 grams gives the percentage of sugar present. A correction should be made for the space occupied by the marc, 26 grams of pulp occupying 0.6 cc.

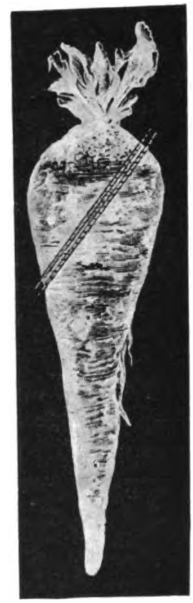

FIG. 3.—Correct position of boring rasp in beet.

The basic lead acetate solution is practically the only one used for clarifying beet solutions for polarization. The clarifying power of this solution depends somewhat upon its basicity. Browne[2] has shown that as the basicity increases the polarization of a raw cane sugar or cane sirup increases. As large an increase will hardly take place in the polarization of a beet, but it shows that care must be taken in the preparation of the basic lead acetate solution, "Three well-defined subacetates have been prepared by the digestion

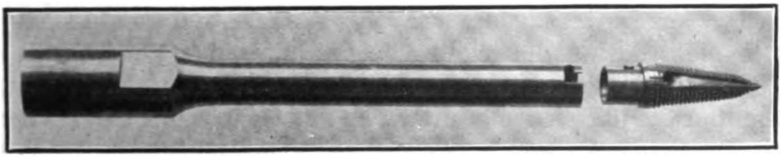

FIG. 4.—Boring rasp.

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[1] See pages 18 and 19.

[2] U. S. Dept. Agr., Bureau of Chemistry Bul. 122, p. 223.

of litharge with normal lead acetate. These are 3PbAc2PbO the one usually prescribed for clarification, PbAcPbO the monobasic, and PbAc2PbO´ the diabasic."[1] Variations in time of digestion, in quantities of litharge used, and in temperature of digestion will result in solutions containing mixtures of all of these.

To prepare this reagent boil 3 parts (by weight) of neutral lead acetate and 1 part of yellow litharge with 10 parts of water until the reaction is completed or the material is practically all dissolved. This generally takes not over half an hour. Cool and dilute the solution with water to a specific gravity of 1.25 or 53.7 Brix. The solution is filtered or allowed to stand until clear. The bottle should be kept tightly corked, as the composition of the solution changes. It may also be prepared by dissolving dry lead subacetate (containing 72.81 per cent of lead) in water until the specific gravity of the solution is 1.25 or 53.7 Brix.

METHODS FOR DETERMINING SUGAR.

Methods for the determination of sugar in the beet may be divided into two general classes, namely, direct and indirect. The former may be subdivided again according to the solvent used and the temperature of extraction. There is only one indirect method and this will be considered first.

INDIRECT METHOD.

The indirect method depends on pressing the juice from the beets, determining the sugar in the juice, and then by a factor calculating the per cent of sugar in the beet. In practice this is accomplished by inclosing the beet cuttings in a jute or cotton cloth, placing the whole in a press, and catching the juice in a vessel large enough to hold it all. The juice is thoroughly mixed, poured into a cylinder, and allowed to stand until the air bubbles have collected at the surface; generally from 20 to 30 minutes is necessary. This foam is brushed aside when the Brix hydrometer is placed in the liquid to obtain a reading on the content of solids. The hydrometer is allowed to come to rest before a reading is made, and should float free of the sides during the reading. After observing the reading and noting the temperature of the solution, the normal or double normal amount of the juice is either drawn up in a sucrose pipette (Spencer's method, *A*, frontispiece) or is weighed. This portion is run into a 100 cc flask with water, and a solution of basic lead acetate added in a sufficient quantity to produce a complete precipitation, but an excess is to be avoided. The flask shown at *B*, frontispiece, is an excellent shape for sugar analysis, because the slanting sides prevent air bubbles from remaining on its surface. The flask is then filled to the mark with water, shaken, filtered, and polarized. The percentage of sugar in the juice thus obtained is multiplied by a factor to obtain the amount of sugar in the beet. This factor corrects for the marc or solid portion of the beet. It is not, however, accurate to determine the marc by the usual method, namely, by washing away the soluble portion of the beet, drying, and weighing, calculating the per cent of solids, subtracting this from 100, and using the figure found for the juice factor. With this procedure the results will be too high for the reason that the marc contains water other than that holding the sugar and no correction is made for it. The marc of beets varies from 4 to 5.5 per cent, with an average of about 4.7 per cent. The juice factor calculated from this figure would be about 95.3, but when the colloidal water or water of marc is considered the figure is from 88 to 95, depending on the condition of the beets and the pressure used. A factor as low as 82 may be obtained if the beets are badly wilted from long standing in a dry climate, such as Colorado. This factor may be determined for a given locality by selecting beets of average condition and finding the per cent of sugar in the juice by the indirect method just given; then on a small portion of the same sample of beets determining the per cent of sugar in the beet by one of the direct methods, and dividing the per cent of sugar in the beet by the per cent of sugar in the juice. Such a factor is applicable to beets of like condition.

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[1] U. S. Dept. Agr., Bureau of Chemistry Bul. 122, p. 223.

The indirect method is used somewhat extensively, for by its use in addition to the percentage of sugar the purity of the juice (the per cent of sugar in the solids of the juice), which is considered by some to be an important factor in beet analysis, is obtained. This method is advantageous in that large quantities of pulp, representing many beets, can be used for pressing; therefore it is very easy to obtain an average sample when the original quantity is large. The main objection to it is that it does not give the sugar in the beet direct, the use of a factor being necessary. This average factor varies somewhat in different localities, ranging from 91.5 to 93, and its use works to the advantage of one sample and to the disadvantage of another. If not much depends on the accuracy of the results, this method can be used, but it can hardly be recommended when exactness is desired.

ERRORS OF THE METHOD.

Errors in the actual determination of the percentage of sugar creep in from the following sources, but with beets of 15 or even 20 per cent sugar content these errors may be within the error of reading or the limits of the sensibility of the instrument:

(1) Use of the wrong normal weight. Most polariscopes that have been purchased during the last five or six years are standardized for

26 grams of sugar, dissolved and made up to 100 true cubic centimeters with water at 20° C., and the polarization made in a 200 mm tube at the same temperature. The old normal weight of 26.048 grams of sugar was for 100 Mohr cubic centimeters, and probably the polarization was to be made at 17.5° C.

(2) Use of the wrong flask, namely, Mohr cubic centimeter flasks in place of true cubic centimeter flasks or vice versa.

(3) Use of old polariscopes whose accuracy has not been checked. A polariscope scale can be checked against pure sugar, but ordinarily it is much better to check against a standardized quartz plate.

(4) Use of sucrose pipette in place of a normal weight. This pipette can be easily standardized and the error due to this source checked, but in general it is better to obtain the normal or double normal amount of juice by weighing.

An error that may amount to several per cent can be introduced by using the same jute cloth for pressing beets when the per cents of sugar in the two samples vary greatly. A large error may also enter from dilution by not removing all of the water used in washing out the press after each sample. Errors in reading the Brix hydrometer are sometimes due to air in the juice and also to the adherence of the hydrometer to the sides of the cylinder. If these chances of error are guarded against, the reading of the per cent of sugar in the juice should be accurate. The following table[1] shows the possible error from the use of a wrong factor:

Variations in results obtained by using different factors.

Sugar in juice.	Sugar in beet calculated by factor—					
	90	91	92	93	94	95
Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
12	10.8	10.92	11.04	11.16	11.28	11.40
13	11.7	11.83	11.96	12.09	12.22	12.35
14	12.6	12.74	12.88	13.02	13.16	13.30
15	13.5	13.65	13.80	13.95	14.10	14.25
16	14.4	14.56	14.72	14.88	15.04	15.20
17	15.3	15.47	15.64	15.81	15.98	16.15
18	16.2	16.38	16.56	16.74	16.92	17.10
19	17.1	17.29	17.48	17.67	17.86	18.05
20	18.0	18.20	18.40	18.60	18.80	19.00
21	18.9	19.11	19.32	19.53	19.74	19.95

Should the juice polarize 20° and a factor of 90 be used when 95 is the correct one, the resulting figure for the sugar in the beet is just 1 per cent too low. If the results by this method are used as a basis for the price paid for beets, the grower would lose the price of an extra per cent of sugar per ton, which varies from 20 to 25, or 33 cents.

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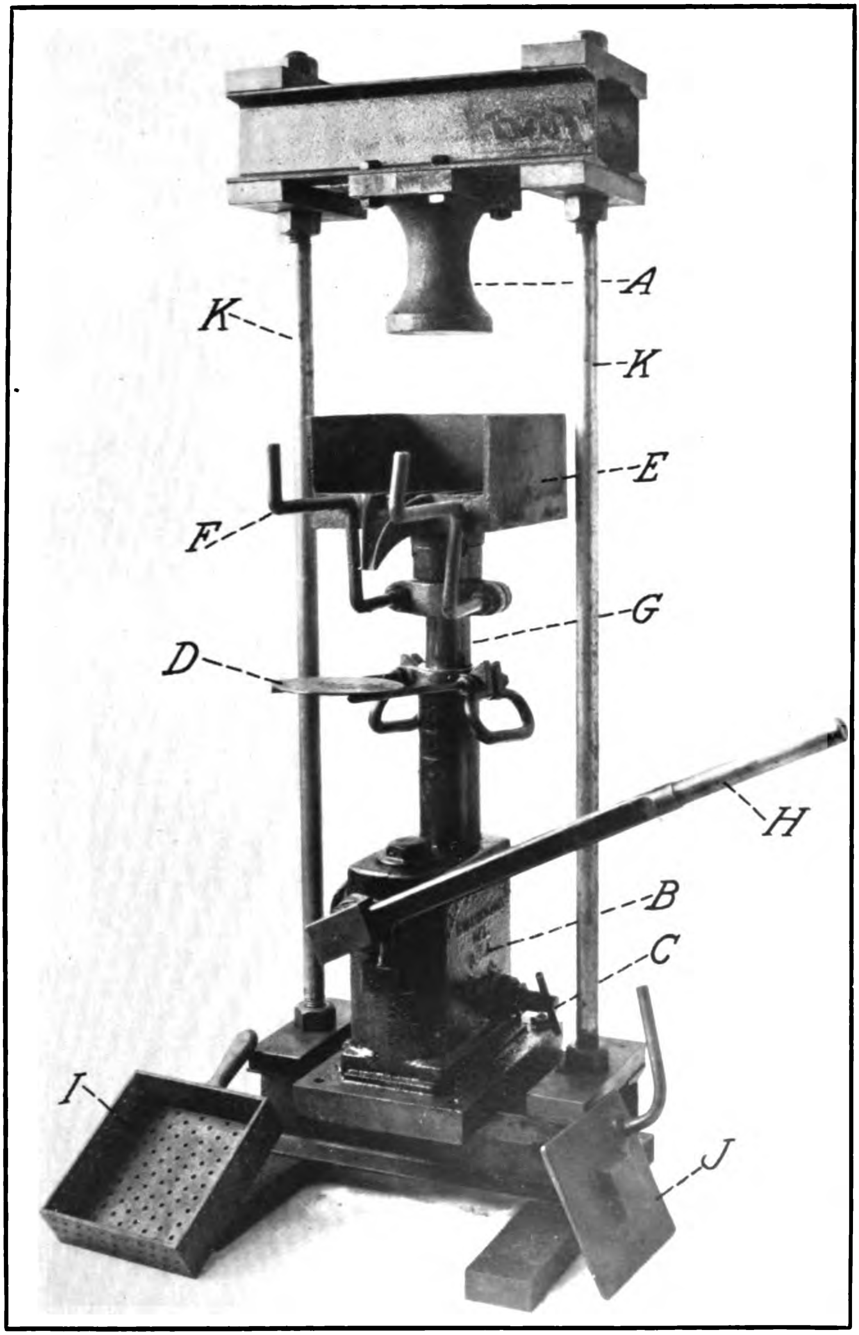
[1] Taken from report of E. E. Ewell, Fifty-sixth Congress, first session, Doc. No. 699, p. 147.



HYDRAULIC PRESS FOR BEET ANALYSIS.

A, Head block. *B*, Hydraulic jack with ram *G*. *C*, Release valve of jack. *D*, Stand for vessel to receive juice. *E*, Receiving box fastened to ram *G*. *H*, Handle for pumping up jack. *I*, *J*, Porous box and cover for beet sample.

HYDRAULIC PRESS.

In Plate I is shown a form of press that has been used successfully for beet pressing by this laboratory. It consists of a hydraulic jack *B* fastened to two pieces of I beam clamped together for the base. Fastened to these clamps are two upright steel rods *K*, and to them are fastened I beams with clamps to hold the head block *A*. Fitted to the ram *G* of the jack is an iron box with lip *E*, also the bent rods *F* and the platform *D*. To operate this press the porous cast-iron pan *I* is placed in the box *E*. A square of jute cloth is then placed in the pan, and the sample of beet cuttings poured into it. The edges of the cloth are folded over and the plate *J* is placed on top. A jar for receiving the juice is placed on the platform *D*, and by means of the handle *H* the jack is jumped up against the head block *A*. When the juice has stopped running, the release valve at *C* is opened and the ram quickly returns to place. The pan *I* can be slid out on the rods *F*, the press cake removed, and a new sample inserted. It is essential to have a pressure gauge on the chamber of the jack, since in analyzing beets by this method the same pressure should be maintained throughout.

DIRECT METHODS.

In the direct methods the per cent of sugar is determined directly on the beet. These methods may be classified according to the solvent used for extraction, namely, water or alcohol, and these may be again divided into extraction in the cold and with heat. So many slight modifications of these different methods are known and used that all of them can not be discussed; only statements of the representative methods can be given.

WATER METHODS.

COLD-WATER DIGESTION METHOD OF PELLET.

Weigh the normal amount of beet cuttings, 26 or 26.048 grams, and transfer to a large-mouth flask (*C*, frontispiece) with a mark at 200.6 cc.[1] Add 5 cc of basic lead acetate solution (see p. 13), shake, and add water up to the shoulder of the flask. Mix the contents by rotating in the hand, and allow to stand 25 minutes in order to expel the air bubbles. Beat down the collected froth with an ether spray and fill the flask to the mark with water. Then shake the contents vigorously, placing the hand over the mouth of the flask, filter, and polarize in a 200 or 400 mm tube after adding a drop of acetic acid.

For this method it is necessary to have the finest divided pulp possible and it is also advisable to have a pear-shaped flask instead of the ordinary round, ball-shaped one in general use, so that the entrained air bubbles may seek the surface and not remain on the sides of the vessel. This method has been much criticized because a finely divided pulp is necessary to prevent an imperfect extraction of the sugar, and also because ordinary shaking will not disengage all of the air bubbles from the pulp, thereby causing the use of a smaller quantity of water to fill the flask to the mark, and thus giving too high results for sugar in the beet.

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[1] Numerous experiments have shown that the marc of the normal weight of average beets occupies about 0.6 cc of space.

COLD-WATER DIGESTION METHOD OF SACHS LE DOCTE.

This is a modification of Pellet's method for the purpose of removing the error resulting from the entrained air. Weigh 26 grams of the fine beet pulp into a tin-lined copper vessel (*D*, frontispiece), add 5 cc of basic lead-acetate solution and 172 cc of water. Put the cover *E* in place and shake the whole vigorously, then allow it to stand for three minutes, remove the cover, filter, and polarize the solution in a 200 mm tube after adding a drop of acetic acid and double the reading, or polarize in a 400 mm tube, in which case the reading gives the per cent of sugar in the beet.

In this method it is assumed that beets contain 95 per cent of juice with an average specific gravity of 1.07. The volume of the juice contained in a normal weight would then be

$$\frac{26\times0.95}{1.07}=23.08 \text{ cc}$$

and (200–23.08 cc) 176.92 cc of water would be necessary to complete the volume to 200 cc. A special pipette (*F*, frontispiece) has been designed to deliver this amount. A quarter turn of the stopcock opens the entrance *G* for the lead acetate, so that 5 cc can be measured, then another quarter turn opens the water entrance *H* to fill the burette to the overflow, and a half turn delivers the whole into the dish.

As in the preceding method, the beet pulp must be very fine, otherwise an imperfect extraction will occur. This method is also open to criticism in that all beets do not contain 95 per cent of juice and all beet juice does not possess a specific gravity of 1.07. It is rightly claimed, however, that the errors introduced by the use of these constants are so small that they will fall within the limits of accuracy of the readings.

HOT-WATER DIGESTION METHOD.

Weigh 52 grams of the beet cuttings and transfer them with water to a large-mouth flask (*C*, frontispiece) of 201.2 cc content; add from 5 to 10 cc of lead subacetate solution, fill the flask to the mark with hot water, and shake. Immerse the flask in a water bath at 80° C. and shake at intervals by rotating. Add water from time to time so that at the end of the heating, about 30 minutes, the water in the flask is a little above the mark. Remove the flask from the water bath and allow it to cool to standard temperature; add a sufficient quantity of concentrated acetic acid to make the solution very slightly acid in reaction (generally less than 0.5 cc is necessary) and a few drops of ether to break the foam; complete the volume. Mix thoroughly, filter, and polarize in a 200 mm tube.

With this method a coarser beet pulp can be used than for the cold-water methods, but over 30 minutes may be necessary for digestion, if very coarse cuttings are used. To obtain correct results, care must be exercised to make up to volume at the standard temperature of 20° C. (if the instrument and flasks are standardized at that temperature) and to digest the beet cuttings with as large a quantity of water as possible. Not over 5 cc in any case should be added during digestion and final cooling to complete the volume. Where smaller quantities of water are used during digestion and then a large quantity of water is added at the last to make to volume, the sugar has not become equally diffused and the results are too low. Differences of over 1 per cent in sugar content are often caused by lack of care in this particular.

HOT-WATER DIGESTION METHOD OF SACHS LE DOCTE.

The procedure in the Sachs Le Docte cold-water extraction method (p. 18) is modified as follows for hot digestion: The weighing and the vessels used are the same, also the quantities of lead subacetate and water are the same, namely, 177 cc. A special rubber disk cover (*I*, frontispiece) is provided for the digestion vessels. Put this in place and after shaking the vessel immerse it in a water bath kept at 80° C. for 30 minutes, or for 25 minutes if the temperature is 85° C. The temperature during extraction should not, however, exceed this figure. Remove the cups and immerse in cold water, bringing the temperature down to 20° C., shake, remove the covers, filter, and polarize, after adding a drop or so of acetic acid.

The chance of error due to contained air or unequal diffusion of the pulp is removed by this method. As in the former case the cuttings need not be so fine as with the cold-water extraction methods.

HERZFELD'S MODIFICATION OF THE SACHS LE DOCTE METHOD.

Instead of the tin-coated copper beakers used in the preceding method, Herzfeld uses an extraction vessel of nickel-plated sheet iron made as shown in figure 5. The vessel is round. He also uses small weighing glasses, holding 26 grams of material, which can be introduced with the beet cuttings into the extraction vessel. These watch glasses are filled to equal weight and numbered consecutively, as are also the extraction vessels. The procedure is as follows:

Weigh 26 grams of the beet pulp on a watch glass and transfer to the extraction vessel, then run in 177 cc of dilute basic lead-acetate solution (5 parts of basic lead-acetate solution, Brix 53.5, to 100 parts of water), shake and place a stopper which has been covered with tinfoil lightly in the opening. Submerge the whole in a water bath at 75° to 80° C. for 30 minutes, shaking intermittently. When all air has been expelled (generally after five minutes), tighten the stopper in the vessel. At the expiration of the time remove and cool. Take out the stopper after shaking thoroughly, filter, and polarize in a 400 mm tube, after addition of a drop of acetic acid to determine the per cent of sugar in the beet.

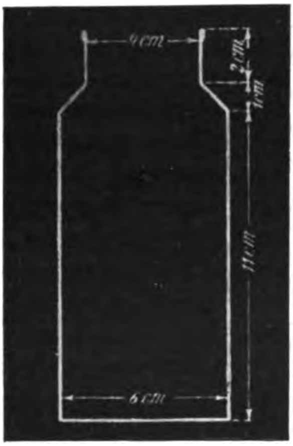

FIG. 5.—Herzfeld's metallic beaker.

This method does not require very fine pulp and is open to few chances of error. It, together with the Sachs Le Docte method, has a decided advantage over the other hot digestion method, in that there is little chance of a loss of sample by the container breaking. When working with glass flasks one is likely to ruin many determinations. For quick work and when many samples are to be run, large heating and cooling vessels can be used and the dishes taken from the one and placed immediately in the other to cool, without risk of losing the determination, thereby saving considerable time. This method has been adopted by the Society of the German Sugar Industry, and with a few modifications in apparatus, but not of procedure, by a number of other sugar associations of Europe.

ALCOHOL METHODS.

The methods using alcohol as the solvent may be divided into two classes—digestion and extraction methods. The former is again divided into hot and cold digestion methods.

COLD ALCOHOL DIGESTION METHOD.

Weigh 52 grams of the beet cuttings and transfer to a flask (having a capacity of 201.2 cc) with 90 per cent alcohol, add 4 cc of lead subacetate solution and shake, then add more 90 per cent alcohol with shaking to remove the air bubbles, complete the volume, allow to stand half an hour, and add alcohol if a decrease in volume is noted. Thoroughly shake the flask and filter, keeping the filter covered to prevent loss by evaporation. Polarize in a 200 mm tube.

With this method very fine pulp is necessary and the greatest care must be exercised to remove all of the air from the beet cuttings to insure an even digestion. For accurate readings the polarization should be made at the standard temperature of the instrument and flasks and allow of no evaporation. This method is but little used.

HOT ALCOHOL DIGESTION METHOD.

The same procedure is followed as in the preceding method up to the point of adding more alcohol. In this method add only enough 90 per cent alcohol to fill the flask three-fourths full. Then connect the flask with a return condenser, place in a water bath, and allow to boil for 20 minutes. Cool the flask and contents to the standard temperature and bring up to the mark with 90 per cent alcohol, shake and allow to stand for awhile, filter, and polarize.

This method does not require so fine a pulp as the former one and is not open to error from entrained air bubbles, but to obtain correct results it must be worked carefully to prevent evaporation and changes of temperature during polarization.

ALCOHOL EXTRACTION METHOD.

The alcohol extraction method has been recognized as the standard method for sugar determination and is the one with which other methods are compared, but its execution is difficult and the results are liable to error if the greatest care is not exercised. For the inexperienced chemist it is not a suitable method, but by familiarizing oneself with its details and difficulties, correct results can be obtained.

A Soxhlet extraction apparatus is necessary for this method. The usual form has been improved by opening the siphon tube with a short tube and cork as shown in the frontispiece, *K*. In this way one can test the progress of the extraction by withdrawing a sample without interfering with the work. The best procedure for this method is to weigh 26 grams of the beet pulp and transfer to a 100 cc flask with about 50 cc of 90 per cent alcohol and from 3 to 5 cc of basic lead-acetate solution. Connect a return condenser to the flask and place in a boiling water bath for from 10 to 15 minutes. Then pour the whole into the extractor, washing out the flask with fresh portions of 90 per cent alcohol. A return condenser is connected with the Soxhlet extractor and also a 100 cc flask, the latter by means of a cork. Add more 90 per cent alcohol until the siphon is started and the lower flask is about three-fourths full. Place the containing flask in a covered water bath held at a heat that will make the alcohol boil freely. Continue the extraction for from one to four hours, or until a test of the alcohol in the extractor gives no color with α-naphthol solution (see p. 22). Remove the flask and add 90 per cent alcohol to the mark after cooling to the standard temperature, shake and filter. Polarize in 200 mm tube.

Care must be exercised to prevent evaporation and changes of temperature and also to use only a minimum amount of basic lead acetate, generally nearer 3 cc than 5 cc, for clarification. By digesting the beet pulp with the alcohol before extraction, the time of extraction is greatly shortened, the pulp becomes thoroughly impregnated with the alcohol, and all air is removed, resulting in a good extraction of the whole material. If the pulp is fine and tends to clog the siphon, alcohol-washed cotton may be used as a plug in the extractor before adding the beet pulp, and a fine mesh screen may be placed over the pulp to keep the whole compact in the extractor.

To determine whether all of the sugar has been extracted or not the following qualitative test is used:

α-naphthol test for sugars.—Add four or five drops of a 20 per cent alcoholic α-naphthol solution to a few drops of the alcohol coming from the extractor and 2 cc of water contained in a small test tube. Shake well, tip the test tube, and allow from 2 to 5 cc of colorless concentrated sulphuric acid to flow down the side of tube; then hold the tube upright, and if sucrose is present a color varying from a faint to a deep violet will be noted at the junction of the two liquids. On shaking, the whole solution becomes a blue violet color. This test is suitable for the results required of it in this work, but it must be remembered that other sugars and substances besides sucrose give this color reaction.

DISCUSSION OF METHODS.

For a number of years the proper methods for determining sugar in the beet have been discussed by sugar chemists, especially as to the relative merits of alcohol and of water extraction. As a general rule German sugar chemists favored alcohol methods, while the French favored water methods. Within the last year a truce has been declared and it is acknowledged that for very accurate or control work the alcohol extraction method should be used, but for general work, one of the hot-water digestion methods will give good results and the analyst is less likely to introduce errors in the manipulation. With the average sample of beets, the two methods when carefully applied will yield duplicate results but in the case of very abnormal beets one method might give higher figures than another. The instantaneous methods (cold extraction) are only suitable with very fine pulp and the results can be depended upon only when they have been checked against a standard method. With these instantaneous methods as many samples of beets can be analyzed per day as by the indirect method, and it is claimed by many that the Sachs Le Docte hot digestion method or the Herzfeld modification will yield as many determinations in a given time as the indirect method, with the same amount of work, provided the laboratory is fitted for the work.

DETAILED ANALYTICAL DATA, 1905–1910.

Sugar-beet analyses, made in the Bureau of Chemistry.

State.	County and town.	Analysis.			Condition of sample.	Serial number.	Date of analysis.
		Average weight.	Sugar in juice.	Purity.			
Alabama	Shelby □	*Ounces.*	*Percent.*				
	Calera	13	13.0	77.6	Fair	7064	Aug. 20, 1909
Arizona	Yuma □						
	Yuma	16	14.6	77.2	Wilted	5626	Aug. 13, 1908
Arkansas	Cleveland □						
	Kingsland	8	11.4	80.3		4374	Aug. 25, 1906
	Craighead □						
	Jonesboro	14	7.40	57.6	Bad	7931	Oct. 17, 1910
	Dallas □						
	Ramsey	16	13.30	82.5	Good	7887	Oct. 3, 1910
	Do	20	13.00	80.8	do	7889	Do.
	Eaglette	14	12.15	79.7	do	7898	Oct. 8, 1910
	Faulkner □						
	Conway	5	15.2	78.8		5151	Nov. 15, 1907
	Pope □						
	Hector	16	13.0	....	Bad	7071	Aug. 21, 1909
California	Imperial □						
	Imperial	11	15.7	83.1	Good	7147	Oct. 3, 1909
	Modoc □						
	Alturas	40	13.75	76.3	do	7966	Oct. 24, 1910
	San Diego □						
	Lakeside	24	21.40	88.7	Fair	7888	Oct. 3, 1910
	Shasta □						
	Cassell	40	14.8	82.2	Good	7203	Oct. 19, 1909
	Siskiyou □						
	Macdoel	16	14.6	85.8	do	5721	Oct. 8, 1908
	Do	16	12.0	79.5	do	5722	Do.
	Do	10	8.0	63.5	Poor red	5725	Oct. 9, 1908
	Do	15	15.3	86.8	Good	5741	Oct. 12, 1908
	Do	4	15.5	85.6	Dry	5778	Oct. 17, 1908
	Do	16	18.1	91.8	Good	5792	Oct. 19, 1908
	Do	25	15.5	79.0	do	7128	Sept. 29, 1909
	Do	24	12.2	79.2	do	7142	Oct. 3, 1909
	Do	24	13.0	78.3	do	7145	Do.
	Do	8	12.7	76.8	do	7150	Do.
	Do	28	14.6	80.6	do	7175	Oct. 9, 1909
Colorado	Boulder □						
	Broomfield	20	10.00	70.6	do	8002	Nov. 1, 1910
	Eagle □						
	Avon	21	14.7	81.5		3760	Nov. 1, 1905
	Do	22	16.2	83.7		3761	Do.
	Bilkin	26	20.2	88.2		3769	Do.
	Eagle	26	15.9	85.0		3770	Do.
	Do	8	18.1	88.3		3771	Do.
	Do	40	20.9	88.7		3778	Nov. 8, 1905
	Avon	24	17.3	84.0		3802	Nov. 14, 1905
	Do	16	18.7	81.7		3811	Nov. 27, 1905
	Gypsum	32	14.6	78.6		3812	Do.
	Do	52	12.9	75.0		3816	Dec. 4, 1905
	Do	48	15.6	82.1		3817	Do.
	Do	20	22.6	89.3		5232	Dec. 9, 1907
	Do	17	18.1	84.1		5248	Dec. 23, 1907
	El Paso □						
	Ramah	16	16.3	83.2	Good	5700	Oct. 5, 1908
	Garfield □						
	Glenwood Springs	36	15.0	84.7		3701	Oct. 5, 1905
	Do	36	10.8	71.1		3702	Do.
	Do	44	15.4	75.5		3703	Do.
	Garfield	24	17.5	86.6		3765	Nov. 1, 1905
	Do	18	14.2	86.6		3766	Do.
	Do	24	11.9	78.8		3767	Do.
	Do	26	19.1	86.8		3768	Do.
	Do	56	17.5	83.5		3777	Nov. 18, 1905
	Do	24	19.2	83.5		3792	Nov. 14, 1905
	Kit Carson □						
	Seibert	....	10.7	66.5	Wilted	5660	Sept. 30, 1908
	Do	15	18.2	84.2	Good	5708	Oct. 6, 1908
	Burlington	19	10.7	72.3	do	5728	Oct. 9, 1908
	Seibert	22	15.0	87.2	do	5745	Oct. 12, 1908
	Do	16	16.4	88.6	Fair	5751	Oct. 13, 1908
	Do	24	9.8	71.2	do	5753	Do.
	Do	16	16.7	85.2	Good	7149	Oct. 3, 1909

Sugar-beet analyses, made in the Bureau of Chemistry—Continued.

State.	County and town.	Analysis.			Condition of sample.	Serial number.	Date of analysis.
		Average weight.	Sugar in juice.	Purity.			
Colorado	Lincoln	*Ounces.*	*Percent.*				
	Limon	18	14.3	81.2	Good	7207	Oct. 19, 1909
	Do	24	9.0	58.8	do	7815	Aug. 6, 1910
	Otero						
	Rocky Ford	24	14.7	77.4	Very bad	7962	Oct. 21, 1910
	Pitkin						
	Aspen	14	17.0	77.8		3803	Nov. 20, 1905
	Routt						
	Steamboat Springs	14	14.1	77.0	Good	7923	Oct. 15, 1910
	Washington						
	Cope	4	14.8	78.7	Very small	5702	Oct. 5, 1908
	Do	15	17.5	82.3	Good	7196	Oct. 15, 1909
	Akron	48	14.0	75.2	do	7329	Nov. 8, 1909
	Do	32	17.4	78.4	Soft	7330	Do.
	Do	38	15.6	81.7	Good	7331	Do.
	Do	48	14.9	79.2	do	7332	Do.
	Do	32	16.2	81.8	do	7333	Do.
	Do	40	15.3	80.1	do	7334	Do.
	Do	45	16.1	82.1	do	7335	Do.
	Do	33	18.3	81.3	do	7336	Do.
	Do	29	20.9	86.4	Soft	7337	Do.
	Do	40	19.0	86.0	do	7338	Do.
	Do	56	18.7	81.3	Good	7339	Do.
	Do	29	18.6	83.4	do	7340	Do.
	Do	36	16.4	82.8	Good, soft	7341	Do.
	Do	28	17.7	80.8	Good	7342	Do.
	Do	37	18.4	84.0	Soft	7343	Do.
	Do	32	19.3	85.4	Good	7344	Do.
	Do	45	16.7	87.0	do	7345	Do.
	Do	26	19.4	85.8	do	7346	Do.
	Do	29	21.6	84.7	do	7347	Do.
	Do	40	19.7	86.3	do	7348	Do.
	Do	48	19.4	84.5		7349	Do.
	Weld						
	La Salle	24	19.9	85.6		3804	Nov. 21, 1905
	Carr	16	17.0	83.7	Fair	5774	Oct. 16, 1908
District of Columbia.	Washington.						
	Do	8	19.2	81.5	Soft	7350	Nov. 8, 1909
	Do	14	20.1	83.3	do	7351	Do.
	Do	28	18.5	75.5	do	7433	Dec. 2, 1909
	Do	22	21.6	78.7	do	7434	Do.
	Do	20	20.7	78.3	do	7436	Do.
	Do	28	13.1	60.1	Rotten, soft.	7437	Do.
	Do	56	17.2	70.1	do	7438	Do.
	Do	52	16.4	70.3	Bad	7439	Do.
	Do	36	17.2	71.1	Soft	7441	Do.
	Do	30	20.0	75.2	Fair	7442	Do.
Georgia	Chattooga						
	Summerville	10	6.2	58.3	Good	7857	Sept. 23, 1910
	Coweta						
	Newman	12	14.0	88.1	do	7903	Oct. 10, 1910
	Floyd						
	Rome	14	8.9	71.6	Good	7176	Oct. 9, 1909
	Greene						
	Penfield	48	7.8	66.6	Soft	5736	Oct. 10, 1908
	Do	17	12.3	80.9	Fair	5735	Do.
Idaho	Blaine						
	Picabo	10	16.5	84.1	Good	8017	Nov. 4, 1910
	Twin Falls						
	Twin Falls	24	20.1	89.5		4381	Oct. 10, 1906
	Do	24	17.7	82.9	Good	8091	Nov. 19, 1910
Illinois	Bond						
	Greenville	20	11.2	74.7	do	8044	Nov. 14, 1910
	Bureau						
	Princeton	48	14.9	76.4	do	7223	Oct. 21, 1909
	Do	21	12.1	76.6	do	7234	Oct. 23, 1909
	Do	20	12.8	77.4	do	7245	Oct. 25, 1909
	Do	48	11.7	72.6	do	7272	Oct. 28, 1909
	Do	32	12.8	76.6	do	7275	Do.
	Do	16	10.8	73.9	do	7290	Nov. 1, 1909
	Do	21	14.9	79.7	do	7294	Do.
	Do	60	10.5	73.2	do	8025	Nov. 8, 1910
	Cook						
	Blue Island	12	19.8	88.0	Fair	5856	Nov. 2, 1908

Sugar-beet analyses, made in the Bureau of Chemistry—Continued.

State.	County and town.	Analysis.			Condition of sample.	Serial number.	Date of analysis.
		Average weight.	Sugar in juice.	Purity.			
Illinois	Henry □	*Ounces.*	*Percent.*				
	Geneseo	32	12.9	80.1		3753	Oct. 31, 1905
	Do	20	10.5	70.2		3754	Do.
	Do	16	12.1	77.7		3755	Do.
	Do	24	11.1	76.1		3756	Do.
	Do	24	12.1	71.6		3757	Do.
	Do	8	14.7	84.0		3758	Do.
	Do	16	11.7	73.3		3759	Do.
	Do	22	13.7	74.6	Withered	4408	Nov. 26, 1906
	Do	16	15.2	78.4	do	4409	Do.
	Do	20	18.3	85.9	do	4410	Do.
	Do	22	16.6	82.6		4413	Do.
	Do	18	17.2	81.2		4414	Do.
	Do	16	19.0			4415	Do.
	Do	16	17.4			4416	Do.
	Do	14	18.9			4417	Do.
	Do	10	20.0			4418	Do.
	Do	10	17.7			4419	Do.
	Do	18	16.2	81.4		4420	Do.
	Do	14	14.7	76.6		4421	Do.
	Do	24	18.4	81.3	Withered	4427	Nov. 27, 1906
	Do	16	18.9	82.9	do	4428	Do.
	Do	38	16.9	81.7	do	4429	Do.
	Do	18	15.9	82.4	do	4430	Do.
	Do	38	13.4	80.7	do	4431	Do.
	Do	16	16.5	81.7	do	4432	Do.
	Do	16	17.8			4434	Do.
	Do	28	16.6	85.1	do	4435	Do.
	Do	16	17.2	86.0	do	4436	Do.
	Do	24	19.1	86.3	do	4437	Do.
	Do	16	17.2	83.0	do	4438	Do.
	Do	20	16.4	80.0	do	4439	Do.
	Do	14	16.7	86.6	do	4440	Do.
	Do	18	14.8	76.3	do	4441	Do.
	Lake □						
	Waukegan	21	13.2	80.0	do	4442	Nov. 31, 1906
	Union □						
	Reynoldsville	37	8.1	64.8	Good	7224	Oct. 21, 1909
	Woodford □						
	Eureka	44	8.9	66.6	Withered	8007	Nov. 2, 1910
Indiana	Huntington □						
	Warren	24	12.4	76.8	Good	7173	Oct. 8, 1909
	Newton □						
	Goodland	36	13.8	79.7	do	8026	Nov. 9, 1910
	Ripley □						
	Versailles	48	9.5	70.0	do	7970	Oct. 27, 1910
	Starke □						
	North Judson	16	16.0	85.1	do	7129	Sept. 29, 1909
Iowa	Allamakee □						
	Harpers Ferry	20	11.5	74.0	do	7146	Oct. 3, 1909
	Do	10	14.7	81.6	do	7228	Oct. 21, 1909
	Fayette □						
	Clermont	39	9.9	70.7	Good-large	7095	Sept. 20, 1909
	Do	52	13.0	78.3	do	7151	Oct. 3, 1909
	West Union	16	13.0	79.1	Good	7235	Oct. 23, 1909
	Do	16	10.4	74.3	do	7246	Oct. 25, 1909
	Clermont	50	12.4	76.1	Good-large	7289	Oct. 30, 1909
	Jefferson □						
	Fairfield	32	12.1	78.6		3737	Oct. 27, 1905
	Lyon □						
	Rock Rapids	5	19.0	90.4	Fair	5719	Oct. 8, 1908
	Palo Alto □						
	Cylinder	21	10.8	69.7		3725	Oct. 27, 1905
	Do	21	15.2	81.3		3726	Do
	Emmetsburg	30	10.2	73.4		3727	Do.
	Do	20	13.1	83.2		3728	Do.
	Do	42	10.3	74.9		3729	Do.
	Do	17	9.7	70.9		3730	Do.
	Do	17	15.3	84.3		3731	Do.
	Do	18	12.3	78.0		3732	Do.
	Do	32	11.7	79.0		3733	Do.
	Do	20	9.5	71.8		3734	Do.
	Do	40	10.7	74.3		3735	Do.
	Osgood	12	12.2	74.2		3738	Do.
	Rodman	26	9.8	69.6		3739	Do.
	Polk □						
	Runnells	15	13.3	74.6		3779	Nov. 9, 1905

Sugar-beet analyses, made in the Bureau of Chemistry—Continued.

State.	County and town.	Analysis.			Condition of sample.	Serial number.	Date of analysis.
		Average weight.	Sugar in juice.	Purity.			
Iowa	Winneshiek □	*Ounces.*	*Percent.*				
	Calmar	24	12.8	73.5	Good	7218	Oct. 21, 1909
	Decorah	48	8.2	63.5	do	7319	Nov. 4, 1909
	Ridgeway	28	12.8	81.0	do	7328	Nov. 8, 1909
	Woodbury □						
	Fallow	24	13.7	79.2		3736	Oct. 27, 1905
	Sioux City	28	13.9	78.5		4893	Oct. 24, 1907
	Do	32	14.0	80.9		4894	Do.
	Do	16	15.2	81.3		4895	Do.
	Do	28	16.8	82.4		4899	Do.
	Do	32	14.6	80.7		4900	Do.
	Do	28	14.4	80.0		4901	Do.
	Do	28	15.6	78.8		4902	Do.
	Do	24	14.7	82.6		4903	Do.
Kansas	Chase □						
	Elmdale	26	8.7	67.4	Withered	5674	Oct. 1, 1908
	Chautauqua □						
	Chautauqua	24	14.6	80.4		4867	Oct. 10, 1907
	Do	24	14.0	80.5		4869	Do.
	Do	12	11.7	76.5		4890	Oct. 22, 1907
	Do	23	11.1	70.6	Slightly wilted.	5030	Nov. 7, 1907
	Do	12	8.6	58.9	Wilted	5031	Do.
	Do	16	9.5	63.3	do	5032	Do.
	Do	33	9.7	69.8	do	5033	Do.
	Do	19	12.6	77.8	do	5034	Do.
	Do	11	13.5	82.3	do	5035	Do.
	Peru	14	7.7	64.7	Withered	5036	Do.
	Chautauqua	9	11.8	71.7		5152	Nov. 15, 1907
	Finney □						
	Garden City	32	15.0	80.2	Fair	5935	Nov. 6, 1908
	Kearny □						
	Deerfield		14.7	82.1	do	5666	Oct. 1, 1908
	Do		9.2	71.9	do	5667	Do.
	Do		10.0	72.5	do	5669	Do.
	Marion □						
	Florence	10	12.4	70.4	Good	8024	Nov. 8, 1910
	Rice □						
	Sterling	26	10.7	76.9	do	5790	Oct. 19, 1908
	Russell □						
	Russell	9	17.9	74.0	Very bad	7967	Oct. 24, 1910
	Do	2	19.3	91.2	Good (dirty)	7997	Oct. 31, 1910
	Wichita □						
	Leoti	24	16.1	82.1	Good	7425	Nov. 29, 1909
Louisiana	Caddo □						
	Shreveport	5	9.5	70.8	do	7863	Sept. 27, 1910
Maryland	Frederick □						
	Frederick	21	17.4	79.8	Wilted	3823	Dec. 16, 1905
	Garrett □						
	Garrett	6	17.6	83.1	Good	8031	Nov. 9, 1910
	Do	22	17.2	87.6	do	8032	Do.
	Do	8	13.5	80.0	do	8034	Do.
	Do	3	10.4	83.1	do	8035	Do.
	Do	17	17.6	90.7	do	8095	Do.
	Do	32	14.8	81.1	do	7933	Oct. 17, 1910
	Mountain Lake Park.	11	16.6	83.5	do	8036	Nov. 9, 1910
	Montgomery □						
	Chevy Chase	19	13.9	77.6	do	7938	Oct. 18, 1910
Michigan	Kalkaska □						
	South Boardman	19	16.4	85.4	Good	7354	Nov. 10, 1909
	Gratiot □						
	Ithaca	16	19.3	83.5		4447	Nov. 2, 1906
	Do	16	20.0	88.2		4448	Do.
	Do	16	20.6	85.1		4449	Do.
	Do	16	22.2	89.1		4450	Do.
	Do	14	19.3			4451	Do.
	Do	18	18.1	84.7		4473	Nov. 19, 1906
	Do	26	16.6	83.4	Slightly moldy.	4491	Dec. 15, 1906
	Do	22	16.8	85.7	Very moldy.	4492	Do.
	Do	24	16.4	82.4	Good	4493	Do.
	Do	16	16.6	79.4	Moldy, dry	4494	Do.
	Do	22	15.2	82.2	Dry	4495	Do.
	Do	26	16.5	81.7	Shriveled and moldy.	4496	Do.
	Do	28	14.5	78.8	Good	4497	Do.
	Do	29	13.9	76.8	do	4498	Do.

Sugar-beet analyses, made in the Bureau of Chemistry—Continued.

State.	County and town.	Analysis.			Condition of sample.	Serial number.	Date of analysis.
		Average weight.	Sugar in juice.	Purity.			
		Ounces.	*Per cent.*				
Michigan	Gratiot	29	16.0	82.0	Good	4499	Dec. 12, 1906
	Ithaca	23	17.3	81.2	do	4500	Do.
	Do	25	16.1	81.7	do	4501	Dec. 15, 1906
	Lenawee						
	Blissfield	10	18.3	83.9		3782	Nov. 14, 1905
	Do	10	18.2	82.7		3783	Do.
	Do	8	18.8	82.5		3784	Do.
	Do	10	19.1	83.8		3785	Do.
	Do	16	19.8	83.2		3786	Do.
	Do	10	19.1	84.7		3787	Do.
	Do	16	18.4	82.5		3788	Do.
	Do	16	17.8	81.3		3789	Do.
	Do	10	18.5	82.6		3790	Do.
	Do	10	17.7	84.7		3791	Do.
	Do	24	9.8	70.3		3795	Nov. 16, 1905
	Do	12	13.1	73.8		3796	Do.
	Do	20	15.1	80.5		3797	Do.
	Do	24	8.4	67.2		3799	Do.
	Monroe						
	Dundee	12	20.9	92.5		3821	Dec. 9, 1905
	Do	20	17.7	87.6		3822	Do.
	St. Clair						
	Marine City	12	18.9	86.7		3772	Nov. 6, 1905
	Do	24	19.0	89.6		3773	Do.
	Do	16	18.2	86.3		3774	Nov. 4, 1905
	Do	16	20.0	86.6		3775	Do.
	Do	12	15.5	81.6		4424	Nov. 9, 1906
	Do	17	17.1	85.6		4480	Nov. 23, 1906
	Do	21	15.4	82.7		4481	Do.
	Tuscola						
	Caro	24	17.1	86.5		4377	Sept. 29, 1906
	Kingston	18	16.3	80.1		4388	Oct. 20, 1906
	Caro	16	14.7	80.6		4389	Oct. 30, 1906
	Kingston	24	16.0	82.5		4391	Oct. 27, 1906
	Do	22	22.2	88.5		4392	Do.
	Do	28	16.9	84.9		4394	Do.
	Colling	16	17.2	86.5	Good	7119	Sept. 28, 1909
	Washtenaw						
	Manchester	16	15.1	85.8		3815	Dec. 1, 1905
	Ypsilanti	34	13.5	81.0		4382	Oct. 11, 1906
	Milan	32	14.4	82.1		4484	Nov. 26, 1906
	Do	26	14.1	81.6		4485	Do.
	Do	15	14.8	83.6		4894	Oct. 24, 1907
	Do	22	18.0	88.7		4939	Nov. 5, 1907
Minnesota	Beltrami						
	Bemidji	9	9.8	66.6	Good	7994	Oct. 29, 1910
Mississippi	Adams						
	Natchez	32	1.3	17.5	do	7072	Aug. 23, 1909
	Do	26	6.4	43.8	Bad, rotten	7073	Do.
	Clay						
	West Point		13.0	78.3		5658	Sept. 29, 1908
	Lincoln						
	Brookhaven	10	11.3	76.4		7092	Sept. 20, 1909
	Do	12	12.0	75.9	Good	7114	Sept. 27, 1909
	Do	6	11.4	76.1	Poor, soft	7130	Sept. 30, 1909
	Do	10	10.9	74.1	Good	7193	Oct. 14, 1909
	Do	20	9.7	72.7	do	7895	Oct. 7, 1910
Missouri	Barton						
	Lamar	2	9.7	70.2	Poor, small	7108	Sept. 21, 1909
	Carter						
	Hunter	11	7.0	63.6	Good	7932	Oct. 17, 1910
	Hickory						
	Quincy	20	8.9	62.6	Poor, soft	7131	Sept. 30, 1909
	Do	19	10.0	69.3	Fair	7232	Oct. 22, 1909
	Howell						
	Willow Springs	8	14.0	84.3	Good	5791	Oct. 19, 1908
	Jasper						
	Jasper	13	12.0	77.3	do	7860	Sept. 26, 1910
	McDonald						
	Powell	9	8.2	65.6	do	7918	Oct. 11, 1910
	Newton						
	Seneca	6	12.1	75.0	Small	7177	Oct. 9, 1909
	Texas						
	Houston	6	9.8	72.9	Good	7891	Oct. 8, 1910
	No county.						
	St. Louis	32	14.0	86.2		6011	Nov. 14, 1908
	Washington						
	Mineral Point	5	14.3	84.9	Fair, small	7120	Sept. 28, 1909
	Wayne						
	Gads Hill		10.3	68.7		5657	Sept. 28, 1908

Sugar-beet analyses, made in the Bureau of Chemistry—Continued.

State.	County and town.	Analysis.			Condition of sample.	Serial number.	Date of analysis.
		Average weight.	Sugar in juice.	Purity.			
Montana	Fergus □	*Ounces.*	*Percent.*				
	Moore	22	16.6	84.8	Good	8043	Nov. 14, 1910
	Lewis and Clark □						
	Augusta	24	16.7	76.8	do	7321	Nov. 4, 1909
	Yellowstone □						
	Billings	5	16.9	87.4	do	7899	Oct. 8, 1910
Nebraska	Boyd □						
	Butte	18	9.2	67.2	do	5732	Oct. 9, 1908
	Johnson □						
	Cook		10.1	69.2	Fair	5671	Oct. 1, 1908
	Lancaster □						
	Havelock	32	12.0	71.4	Good	7326	Nov. 8, 1909
	Lincoln □						
	North Platte	21	9.5	69.8	Fair	5951	Nov. 9, 1908
	Do	18	9.9	69.2	do	5952	Do.
	Do	17	9.2	66.1	do	5953	Do.
	Scotts Bluff □						
	Morrill	21	15.9	84.4	Good	7293	Nov. 1, 1909
	Do	24	18.7	85.0	Fair	7424	Nov. 29, 1909
	Do	17	16.3	82.5	Good	7426	Do.
Nevada	Churchill □						
	Fallon	24	15.5	91.7	do	5809	Oct. 23, 1908
	Hazen	16	18.3	87.1	do	5949	Nov. 7, 1908
	Fallon	40	17.9	84.4	Fair	5992	Nov. 10, 1908
	Do	21	15.0	82.8	Good	6004	Nov. 12, 1908
	Do	9	17.4	83.2	Fair	6022	Nov. 17, 1908
	Do	24	16.4	83.2	Good	7148	Oct. 3, 1909
	Do	11	16.1	83.6	do	7188	Oct. 12, 1909
	Do	24	16.7	81.0	do	7277	Oct. 29, 1909
	Do	24	16.7	84.2	do	7278	Do.
	Do	19	19.5	89.0	do	7279	Do.
	Do	21	18.9	87.5	do	7280	Do.
	Do	17	20.3	91.4	do	7281	Do.
	Do	20	19.0	90.0	do	7282	Do.
	Do	27	21.0	92.5	do	7283	Do.
	Do	20	20.0	90.5	do	7284	Do.
	Do	24	19.6	89.5	do	7285	Do.
	Do	24	19.6	89.5	do	7286	Do.
	Hazen	48	16.6	82.6	do	7394	Nov. 19, 1909
New Jersey	Hudson □						
	Bayonne	16	8.3	63.5	Fair	7091	Sept. 20, 1909
New Mexico	Chaves □						
	Roswell	13	19.5	87.3		4619	Jan. 16, 1907
	Do	10	19.2	84.2		4620	Do.
	Do	10	20.4	86.4		4621	Do.
	Do	13	17.7	81.6		4622	Do.
	Colfax □						
	Cimarron	28	17.5	80.4	Wilted	5207	Nov. 29, 1907
	Do	18	16.1	84.1	Fair	5690	Oct. 3, 1908
	Do	28	16.0	85.1	Good	5703	Oct. 5, 1908
	Curry □						
	Texico	25	19.7	92.1	do	5946	Nov. 7, 1907
	St. Vrain	28	19.1	86.0	Fair	5948	Do.
	Eddy □						
	Artesia	17	9.5	75.4	Red, soft	5795	Oct. 19, 1908
	Lincoln □						
	Carrizozo	15	18.5	87.2		5255	Dec. 30, 1907
	Mora □						
	La Cueva	16	19.7	84.9		5048	Nov. 8, 1907
	Do	16	18.2	80.9		5049	Do.
	Do	16	22.3	86.9		5238	Dec. 13, 1907
	Do	24	21.7	86.9		5239	Do.
	Do	19	17.0	78.7	Good	6033	Nov. 20, 1908
	Do	24	18.8	84.6	Fair	6082	Dec. 28, 1908
	Levy	18	12.0	73.9	Good	7187	Oct. 12, 1909
	Do	16	17.5	86.9	do	7323	Nov. 4, 1909
	Wagon Mound	30	16.6	82.5	do	7353	Nov. 9, 1909
	Quay □						
	Nara Visa	6	10.7			6042	Nov. 23, 1908
	Roosevelt □						
	Portales	18	15.2	82.3		4386	Oct. 12, 1906
	Claudell	7	20.9	88.6	Fair	5779	Oct. 17, 1908
	Upton	9	18.5	88.5	Poor	5815	Oct. 26, 1908
	Elida	17	19.4	95.1	Fair	5838	Oct. 27, 1908
	Portales	12	10.9	72.6	Good	7153	Oct. 5, 1909

Sugar-beet analyses, made in the Bureau of Chemistry—Continued.

State.	County and town.	Analysis.			Condition of sample.	Serial number.	Date of analysis.
		Average weight.	Sugar in juice.	Purity.			
New Mexico....	San Miguel	*Ounces.*	*Percent.*				
	East Las Vegas...	18	15.7	78.5		4908	Oct. 28, 1907
	Do............	36	18.2	79.8		5058	Nov. 8, 1907
	Las Vegas.........	12	20.6	90.4		5079	Nov. 11, 1907
	Do............	60	18.3	78.5		5080	Do.
	Rociada...........	16	18.7	81.3		5081	Do.
	San Jose..........	46	13.6	70.5		5082	Do.
	Las Vegas.........	10	20.8	88.8		5085	Nov. 12, 1907
	East Las Vegas...	22	18.1	80.4		5149	Nov. 14, 1907
	Do............	15	22.2	90.2		5206	Nov. 25, 1907
	Las Vegas.........	8	22.1		Withered...	5240	Dec. 13, 1907
	Do............	4	11.9	76.0	Withered, small.	5241	Do.
	East Las Vegas...	16	14.3	85.6	Fair.........	6052	Nov. 24, 1908
	Los Alamos.......	22	10.0	80.6	Poor........	6056	Do.
	East Las Vegas...	32	13.6	77.3	Good........	7248	Oct. 26, 1909
	Las Vegas.........	56	14.8	77.3	.....do.......	7395	Nov. 19, 1909
	Do............	17	16.8	77.0	Withered...	8156	Dec. 8, 1910
	Santa Fe						
	Otto..............	24	16.7	83.5	Fair.........	5688	Oct. 3, 1908
	Do............	9	16.9	86.2	Small.......	5695	Oct. 5, 1908
	Do............	34	17.1	86.3	Fair.........	5698	Do.
	Stanley...........	32	16.1	83.0	Good........	5699	Do.
	Otto..............	11	15.6	83.9	.....do.......	5701	Do.
	Stanley...........	15	15.7	86.7	.....do.......	5704	Do.
	Otto..............	17	16.5	89.2	.....do.......	5706	Oct. 6, 1908
	Hyer..............	35	16.0	84.6	.....do.......	5707	Do.
	Otto..............	13	13.8	80.0	Fair.........	5709	Do.
	Hyer..............	12	19.5	85.9	Good........	5713	Do.
	Otto..............	13	17.4	87.8	.....do.......	5720	Oct. 8, 1908
	Do............	30	15.7	85.3	.....do.......	5726	Oct. 9, 1908
	Stanley...........	11	14.2	82.5	.....do.......	5730	Do.
	Do............	13	18.2	91.0	Fair.........	5733	Do.
	Otto..............	52	10.5	71.9	Good........	5752	Oct. 12, 1908
	Stanley...........	19	17.0	85.0	Fair.........	5783	Oct. 17, 1908
	Do............	16	21.5	95.1	.....do.......	5784	Do.
	Do............	27	15.9	91.0	Good........	5800	Oct. 20, 1908
	Otto..............	35	11.9	84.3	.....do.......	5808	Oct. 23, 1908
	Stanley...........	15	21.9			5934	Nov. 6, 1908
	Sierra						
	Arrey.............	24	12.9	72.5	Soft.........	7231	Oct. 22, 1909
	Taos						
	Taos..............	22	17.0	73.8	Withered...	4464	Nov. 14, 1906
	Torrance						
	Moriarty..........	28	17.3	84.0		5675	Oct. 2, 1908
	Do............	19	17.4	80.2	Withered...	5676	Do.
	Do............	18	18.7	85.0	Fair.........	5689	Oct. 3, 1908
	McIntosh..........	24	15.7	86.2	Good........	5696	Oct. 5, 1908
	Moriarty..........	24	17.6	85.4	.....do.......	5697	Do.
	Do............	12	16.9	84.5	.....do.......	5710	Oct. 6, 1908
	Do............	11	18.5	81.0	.....do.......	5727	Oct. 9, 1908
	Do	10	17.3	88.3	.....do.......	5731	Do.
	Do............	12	16.0	94.7	.....do.......	5742	Oct. 12, 1908
	Do............	18	16.5	83.4	Fair.........	5746	Do.
	Estancia..........	20	11.0	72.6	.....do.......	5747	Do.
	Do............	33	13.2	81.6	Good........	5749	Do.
	Moriarty..........	20	16.0	82.8	.....do.......	5750	Oct. 13, 1908
	Do............	17	15.0	80.2	.....do.......	5754	Oct. 12, 1908
	Do............	16	19.8	73.0	Fair.........	5772	Oct. 15, 1908
	Do............	29	13.2	83.5	Good........	5793	Oct. 19, 1908
	Do............	64	16.5	80.4	.....do.......	5796	Do.
	Do............	4	13.5	85.4	Very small..	5812	Oct. 24, 1908
	Do............	24	16.9	78.2	Fair.........	5901	Nov. 4, 1908
	Do............	24	15.6	80.0	.....do.......	5903	Do.
	Union						
	Amistad..........	4	22.0	93.6	Very small..	5839	Oct. 28, 1908
	Clayton...........	12	17.0	86.2	Good........	7195	Oct. 15, 1909
	Do............	16	16.3	86.2	.....do.......	7202	Oct. 19, 1909
	Do............	9	14.5	75.5	.....do.......	7221	Oct. 21, 1909
New York......	Erie						
	Buffalo...........	12	13.3	80.0	.....do.......	8008	Nov. 2, 1910
	Kings						
	Brooklyn.........	8	15.7	86.9	.....do.......	7862	Sept. 26, 1910

Sugar-beet analyses, made in the Bureau of Chemistry—Continued.

State.	County and town.	Analysis.			Condition of sample.	Serial number.	Date of analysis.
		Average weight.	Sugar in juice.	Purity.			
North Carolina	Ashe	*Ounces.*	*Percent.*				
	River Side	24	15.0	80.2	Good	7322	Nov. 4, 1909
	Beaufort						
	Washington	16	16.4	87.7	Wilted	4864	Oct. 10, 1907
	Do	16	14.0	83.3	do	4865	Do.
	Aurora	7	9.5	76.5		4885	Oct. 15, 1907
	Washington	36	11.6	75.3	Good	5620	July 31, 1908
	Do	16	7.8	70.0	Small	5655	Sept. 26, 1908
	Buncombe						
	Asheville	13	16.2	84.8		4936	Oct. 30, 1907
	Davie						
	Advance	5	11.0	78.5	Poor, small	7107	Sept. 21, 1909
	Guilford						
	Julian	32	11.8	82.5		4879	Oct. 14, 1907
	Haywood						
	Canton	24	14.2	82.7	Good	7974	Oct. 28, 1910
	Moore						
	Carthage	8	15.1	85.1	do	7144	Oct. 3, 1909
	Robeson						
	Maxton	16	12.5	83.3	Wilted	4866	Oct. 10, 1907
	Do	18	9.6	68.1		4868	Do.
	Watauga						
	Sands	18	17.6	86.5	Good	7204	Oct. 19, 1909
North Dakota	Barnes						
	Valley City	52	14.8	76.7	do	7992	Oct. 29, 1910
	Cavalier						
	Sarles	7	14.8	80.8	do	8000	Nov. 1, 1910
	Sargent						
	Cogswell	28	9.0	67.0	do	7891	Sept. 30, 1910
	Richland						
	Great Bend	28	11.4	68.8		3723	Oct. 18, 1905
	Do	44	11.5	68.2		3724	Do.
	Do	24	11.3	73.9		3749	Oct. 31, 1905
	Do	32	12.6	67.2		3750	Do.
	Do	48	14.7	78.4		3751	Do.
	Do	32	18.9	86.7		3752	Do.
	Walsh						
	Grafton	10	15.7	83.0	Good	7995	Oct. 29, 1910
	Wells						
	Hurdsfield	11	18.0	82.2	Fair	5687	Oct. 3, 1908
	Do	12	14.3	80.3	Good	5794	Oct. 19, 1908
	Do	24	16.2	85.7	do	5799	Oct. 20, 1908
	Do	17	16.6	86.5	do	5816	Oct. 26, 1908
	Do	10	16.0	94.1	Fair	5842	Oct. 27, 1908
	Do	20	16.1	90.9	Hard	5931	Nov. 5, 1908
	Do	15	8.2	65.5	Poor	5954	Nov. 9, 1908
Ohio	Brown						
	Ripley	16	10.0	73.0	Fair	6072	Dec. 9, 1908
	Columbiana						
	East Liverpool	24	10.4	69.3	Good	7919	Oct. 12, 1910
	Guernsey						
	Birds Run	13	14.6	80.6	do	8014	Nov. 3, 1910
	Hocking						
	Logan	24	16.8	84.6	do	7154	Oct. 6, 1909
	Marion						
	Marion	10	15.9	85.8	Fair	7233	Oct. 23, 1909
	Ottawa						
	Oak Harbor	20	17.5	84.9		4490	Dec. 12, 1906
	Paulding						
	Paulding	35	17.9	82.6		4407	Oct. 27, 1906
	Do	37	16.0	78.8		4422	Nov. 9, 1906
	Do	36	16.8	78.9		4423	Do.
	Haviland	12	21.2	88.0		4905	Oct. 28, 1907
	Do	8	21.0	86.4		4906	Do.
	Do	16	19.4	89.8		4907	Do.
	Do	18	19.3	89.8		5050	Nov. 8, 1907
	Do	22	16.8	88.2		5051	Do.
	Do	18	17.9	90.4		5052	Do.
	Do	15	18.4	89.3		5053	Do.
	Do	14	19.8	90.8		5054	Do.
	Do	12	19.8	91.7		5055	Do.
	Do	13	17.2	88.2		5056	Do.
	Do	13	16.9	89.2		5057	Do.
	Broughton	9	21.0	81.6	Dry, wilted	5158	Nov. 20, 1907
	Haviland	16	21.7	82.2	Badly wilted	5160	Nov. 22, 1907
	Van Wert						
	Scott	8	19.4	87.4	Wilted	5159	Do.
	Do	20	17.0	85.6		5211	Dec. 2, 1907
	Williams						
	Bryan	8	16.9	83.3	Very dry	4617	Jan. 9, 1906

Sugar-beet analyses, made in the Bureau of Chemistry—Continued.

State.	County and town.	Analysis.			Condition of sample.	Serial number.	Date of analysis.
		Average weight.	Sugar in juice.	Purity.			
Oklahoma	Beaver □	*Ounces.*	*Percent.*				
	Floris	48	14.9	86.6	Fair	5849	Oct. 31, 1908
	Do	26	14.2	79.7	Good	6003	Nov. 12, 1908
	Do	20	15.1	79.1	Fair	6012	Nov. 14, 1908
	Do	34	15.5	81.5	do	6014	Do.
	Do	11	14.1	80.6	do	6019	Nov. 16, 1908
	Do	8	16.7	83.9	do	6035	Nov. 20, 1908
	Do	7	17.4	84.6	Good	7201	Oct. 19, 1909
	Do	10	15.0	74.2	do	7209	Do.
	Do	6	17.5	78.5	do	7392	Nov. 19, 1909
	Do	20	13.4	77.6	Fair	8160	Dec. 13, 1910
	Carter □						
	Ardmore	24	12.9	91.9	Good	7327	Nov. 8, 1909
	Comanche □						
	Chattanooga	13	11.0	68.7	do	7427	Nov. 29, 1909
	Harmon □						
	Vinson	7	16.2	78.2	Fair	7109	Sept. 24, 1909
	Kiowa □						
	Mountain Park	22	11.6	78.6	Good	7861	Sept. 26, 1910
	Swanson □						
	Snyder	24	14.9	81.6	do	7836	Sept. 19, 1910
	Do	9	16.6	89.8	do	8020	Nov. 8, 1910
	McIntosh □						
	Hoffman	15	12.7	91.3	Good, soft	7106	Sept. 21, 1909
	Grayson	10	5.8	50.0	do	7185	Oct. 12, 1909
	Muskogee □						
	Braggs		11.5	76.1		5668	Oct. 1, 1908
	Do	19	10.1	72.7	Good	7094	Sept. 20, 1909
	Do	26	13.6	75.8	do	7113	Sept. 27, 1909
	Wybark	12	14.0	80.7	do	7115	Do.
	Braggs	16	10.9	71.2	Fair	7117	Sept. 28, 1909
	Do	32	6.2	50.8	do	7152	Oct. 5, 1909
	Council Hill	2	13.2	77.0	Fair, small	7184	Oct. 11, 1909
	Okfuskee □						
	Castle	16	8.5	64.9	do	7229	Oct. 21, 1909
	Roger Mills □						
	McArthur	26	11.9	74.4		4887	Oct. 18, 1907
	Grimes	4	16.5	83.5	Good	7199	Oct. 16, 1909
	Washita □						
	Port	26	13.4	78.8		5150	Nov. 15, 1907
	Woods □						
	Alva	20	20.8	81.1	Dry	5237	Dec. 11, 1907
Oregon	Crook □						
	Tumalo	16	17.0	85.0	Very dry	3704	Oct. 12, 1905
	Do	16	13.0	76.9		3705	Oct. 16, 1905
	Klamath □						
	Klamath Falls	32	21.9	92.0	Wilted	5037	Nov. 8, 1907
	Do	15	15.7	77.7		5083	Nov. 11, 1907
	Merrill	30	16.5	79.6		5210	Dec. 2, 1907
	Klamath Falls	16	20.6	90.7	Fair	5748	Oct. 12, 1908
	Do	10	16.5	86.8	do	5775	Oct. 17, 1908
	Do	24	16.3	81.0	do	6015	Nov. 14, 1908
	Do	37	18.3	76.0	Withered	8029	Nov. 9, 1910
	Merrill	18	14.5	76.0	Good	7896	Oct. 7, 1910
	Do	10	14.5	80.8	do	7897	Oct. 8, 1910
	Do	32	14.5	77.1	Bad	7969	Oct. 27, 1910
	Do	16	18.0	87.9	Good	8009	Nov. 3, 1910
	Do	16	18.8	87.8	do	8011	Do.
	Do	13	19.3	87.7	do	8012	Do.
	Do	13	18.3	88.8	do	8013	Do.
	Do	16	20.4	88.7	do	8015	Do.
	Do	26	13.4	74.7	do	8016	Do.
	Do	35	17.3	76.1	Withered	8027	Nov. 9, 1910
	Do	28	15.9	74.7	do	8028	Do.
	Do	12	18.5	88.0	Good	8041	Nov. 14, 1910
	Do	24	16.0	82.0	do	8042	Do.
	Do	20	18.5	88.3	do	8048	Nov. 19, 1910
	Lincoln □						
	Elk City	32	12.8	67.9	Withered	4446	Nov. 30. 1906
Pennsylvania	Center □						
	State College	12	14.9	81.4	Good	7181	Oct. 11, 1909
	Lancaster □						
	Manheim		16.9	84.0	Fair	5665	Oct. 1, 1908
	Philadelphia □						
	Philadelphia		10.3	71.5	Small	5661	Sept. 30, 1908
	Potter □						
	Ulysses	3	16.1	79.7		5208	Dec. 2, 1907
	Do	18	17.5	91.6	Good	5717	Oct. 7, 1908

Sugar-beet analyses, made in the Bureau of Chemistry—Continued.

State.	County and town.	Analysis.			Condition of sample.	Serial number.	Date of analysis.
		Average weight.	Sugar in juice.	Purity.			
South Dakota	Butte □	*Ounces.*	*Percent.*				
	Vale	16	18.0	75.3	Pithy	4903	Oct. 24, 1907
	Stanley □						
	Philip	14	19.5	83.7	Very soft	7428	Nov. 30, 1909
Tennessee	Coffee □						
	Tullahoma	12	8.7	69.6	Fair	7096	Sept. 21, 1909
	Do	5	11.9	77.1	Good	7222	Oct. 21, 1909
	Do	17	8.7	66.4	do	7247	Oct. 25, 1909
	Do	22	13.8	76.7	Fair	7429	Nov. 30, 1909
	Dickson □						
	Tennessee City	19	7.6	62.2	Good	7088	Sept. 16, 1909
	Franklin □						
	Winchester	24	10.8	75.5	Fair	7172	Oct. 8, 1909
	Grainger □						
	Rutledge	21	13.6	81.7	Good	7236	Oct. 23, 1909
	Hamblen □						
	Morristown	16	10.1	73.7	do	7118	Sept. 28, 1909
	Do	12	10.9	76.2	Fair	7288	Oct. 30, 1909
	Hickman □						
	Vernon	21	8.0	61.6	Poor	7090	Sept. 20, 1909
	Do	20	9.3	73.2	Good	7093	Do.
	Do	7	10.2	71.3	do	7393	Nov. 19, 1909
	Do	40	6.9	60.0	do	7999	Oct. 31, 1910
	Humphreys □						
	McEwen	4	13.9	83.2	Fair	7089	Sept. 17, 1909
	Do	9	14.7	85.6	Good	7886	Oct. 4, 1910
	Warren □						
	McMinnville	22	5.9	47.2	Fair	7205	Oct. 19, 1909
Texas	Armstrong □						
	Goodnight	22	15.4	84.2	Good	7902	Oct. 8, 1910
	Brown □						
	Blanket	19	13.1	74.0	do	6038	Nov. 20, 1908
	Carson □						
	Groom	60	12.8	76.4		3706	Oct. 16, 1905
	Do	10	13.3	74.5		3707	Do.
	Do	56	12.8	71.5		3708	Do.
	Do	64	13.2	73.3		3710	Do.
	Do	35	14.0	80.5		5233	Dec. 10, 1907
	Cass □						
	Avinger	20	8.3	64.9	Good	8023	Nov. 8, 1910
	Castro □						
	Summerfield	6	15.9			6043	Nov. 23, 1908
	Comanche □						
	Comanche	18	13.0	77.4	Soft	6037	Nov. 20, 1908
	Dallam □						
	Dalhart	24	14.2	81.6	Small, very dry.	3712	Oct. 18, 1905
	Do	12	13.9	79.1	Very dry	3713	Do.
	Do	16	13.0	73.6	Dry	3714	Do.
	Do	12	14.0	79.3	do	3715	Do.
	Do	19	15.3	81.2		5028	Nov. 7, 1907
	Do	12	16.5	86.0	Good	6010	Nov. 13, 1908
	Texline	26	12.0	71.4	do	7182	Oct. 11, 1909
	Do	16	14.2	77.1	do	7183	Do.
	Do	16	12.5	70.5	do	7273	Oct. 28, 1909
	Do	16	15.4	79.6	do	7291	Nov. 1, 1909
	Dallas □						
	Dallas	16	3.0	35.7		5084	Nov. 11, 1907
	Donley □						
	Jericho	44	10.3	70.3		3709	Oct. 16, 1905
	Do	32	13.8	74.6		4444	Nov. 31, 1906
	Clarendon	31	15.5	77.4		4465	Nov. 14, 1906
	Do	25	12.6	73.3		5153	Nov. 19, 1907
	Erath □						
	Stephenville	20	12.2	70.1	Fair	6039	Nov. 20, 1908
	Dublin	35	11.4	72.1	do	6040	Do.
	Fannin □						
	Honey Grove	20	17.8	82.9	do	7864	Sept. 27, 1910
	Do	12	14.2	80.8	Good	7872	Oct. 1, 1910
	Do	28	15.2	80.7	do	7873	Sept. 30, 1910
	Do	28	17.3	84.7	do	7874	Do.
	Do	16	13.4	70.5	do	7890	Oct. 3, 1910
	Do	13	11.6	75.5	do	7941	Oct. 19, 1910
	Gray □						
	McLean	16	10.0	70.2	Very dry	3721	Oct. 18, 1905
	Do	20	7.9	58.5	do	3722	Do.
	Do	40	11.9	77.8		4375	Sept. 25, 1906
	Do	40	16.3	82.9		4379	Oct. 8, 1906
	Do	26	15.3	74.7		4445	Nov. 31, 1906
	Do	16	14.2	83.5	Fair	5850	Oct. 31, 1908

Sugar-beet analyses, made in the Bureau of Chemistry—Continued.

State.	County and town.	Analysis.			Condition of sample.	Serial number.	Date of analysis.
		Average weight.	Sugar in juice.	Purity.			
Texas..........	Hamilton □	*Ounces.*	*Per cent.*				
	Hico..........	36	13.3	79.3	Good........	8022	Nov. 8,1910
	Hartley □						
	Channing..........	7	10.7	72.3	....	3716	Oct. 18,1905
	Do..........	20	13.6	78.6	Very dry....	3717	Do.
	Do..........	12	7.5	60.0	.....do.......	3718	Do.
	Do..........	20	13.6	76.8	.....do.......	3719	Do.
	Do..........	20	13.0	73.0	.....do.......	3720	Do.
	Hartley..........	32	11.5	69.3	Withered...	4395	Oct. 27,1906
	Channing..........	33	17.9	79.4	.....do.......	4443	Nov. 31,1906
	Do..........	30	14.3	72.9	.....do.......	4453	Nov. 5,1906
	Hunt □						
	Cash..........	11	12.2	77.2	Fair........	6018	Nov. 16,1908
	Jeff Davis □						
	Valentine..........	16	18.0	86.5	.....do.......	6051	Nov. 24,1908
	Kaufman □						
	Terrell..........	10	10.6	66.1	.....do.......	7922	Oct. 14,1910
	Oldham □						
	Wildorado..........	16	16.5	83.3	.....do.......	6069	Dec. 5,1908
	Parmer □						
	Bovina..........	28	13.8	72.2	....	4387	Oct. 19,1906
	Do..........	6	18.7	89.5	Good........	7324	Nov. 4,1909
	Polk □						
	Onalaska..........	33	8.3	53.2	Fair........	7040	July 26,1909
	Potter □						
	Amarillo..........	40	11.1	70.0	....	3741	Oct. 31,1905
	Do..........	36	16.7	82.8	....	3742	Do.
	Do..........	36	17.0	82.1	....	3743	Do.
	Do..........	32	12.8	78.8	....	3744	Do.
	Do..........	24	11.3	76.0	....	3745	Do.
	Do..........	16	16.3	84.0	....	3746	Do.
	Do..........	28	16.0	83.1	....	3747	Do.
	Do..........	32	18.3	87.7	....	3748	Do.
	Do..........	16	11.2	75.2	....	4380	Oct. 8,1906
	Do..........	21	16.7	81.8	....	4463	Nov. 13,1906
	Do..........	27	15.4	73.2	....	4884	Oct. 15,1907
	Do..........	7	16.5	80.5	Healthy.....	4937	Oct. 31,1907
	Do..........	6	20.1	85.5	.....do.......	4938	Do.
	Do..........	27	13.1	76.7	....	5059	Nov. 9,1907
	Do..........	19	14.4	81.8	Good........	5994	Nov. 10,1908
	Do..........	10	14.3	75.2	.....do.......	7356	Nov. 16,1909
	Do..........	16	14.9	77.2	.....do.......	7357	Do.
	Do..........	16	15.6	78.7	.....do.......	7358	Do.
	Do..........	18	15.8	78.6	.....do.......	7359	Do.
	Do..........	16	15.3	79.2	.....do.......	7360	Do.
	Do..........	16	16.4	79.2	.....do.......	7361	Do.
	Do..........	18	15.5	79.0	.....do.......	7362	Do.
	Do..........	16	17.5	81.3	.....do.......	7363	Do.
	Do..........	16	17.9	83.2	.....do.......	7364	Do.
	Do..........	16	18.4	84.0	.....do.......	7365	Do.
	Do..........	10	18.2	78.1	.....do.......	7366	Do.
	Do..........	13	16.4	77.0	.....do.......	7367	Do.
	Do..........	13	17.0	79.5	.....do.......	7368	Do.
	Do..........	10	17.4	77.2	.....do.......	7369	Do.
	Do..........	10	18.1	79.4	.....do.......	7370	Do.
	Do..........	16	15.8	75.9	.....do.......	7371	Do.
	Do..........	10	17.4	80.2	.....do.......	7372	Do.
	Do..........	13	17.7	77.6	.....do.......	7373	Do.
	Do..........	16	16.7	77.7	.....do.......	7374	Do.
	Do..........	16	16.7	79.1	.....do.......	7375	Do.
	Randall □						
	Canyon..........	25	15.7	89.2	.....do.......	5744	Oct. 12,1908
	Reeves □						
	Pecos..........	18	16.4	75.2	Fair........	6077	Dec. 28,1908
	Runnels □						
	Ballinger..........	12	14.0	79.1	Fair........	6055	Nov. 24,1908
	Scurry □						
	Hermleigh..........	32	9.9	75.6	....	5680	Oct. 2,1908
	Do..........	48	13.1	82.3	Poor........	5681	Do.
	Do..........	11	15.6	81.2	Dry........	5777	Oct. 17,1908
	Pyron..........	16	16.5	80.0	Fair........	5987	Nov. 10,1908
	Winston..........	32	14.2	79.3	.....do.......	6028	Nov. 17,1908
	Sherman □						
	Stratford..........	16	16.3	74.0	Wilted......	4502	Dec. 28,1906
	Do..........	11	16.0	74.3	.....do.......	4503	Do.
	Tarrant □						
	Keller..........	5	5.0	51.0	Small,soggy.	5729	Oct. 9,1908
	Fort Worth..........	13	10.4	69.8	Fair........	6036	Nov. 20,1908

Sugar-beet analyses, made in the Bureau of Chemistry—Continued.

State.	County and town.	Analysis.			Condition of sample.	Serial number.	Date of analysis.
		Average weight.	Sugar in juice.	Purity.			
Texas	Van Zandt	*Ounces.*	*Per cent.*				
	Canton	16	9.0	79.6	Poor	5743	Oct. 12, 1908
	Victoria						
	Victoria	56	7.3	52.9	Wilted and moldy.	5603	July 1, 1908
	Wheeler						
	Ramsdell	16	16.5	88.2	Good	6025	Nov. 17, 1908
	Wilbarger						
	Vernon	32	10.9	79.0		4371	Sept. 24, 1906
	Do	32	10.2	73.9		4372	Do.
	Do	20	11.5	78.3		4373	Do.
	Do	16	13.4	74.6	Withered	4452	Nov. 3, 1906
Utah	Juab						
	Nephi	9	11.9	75.1	Good	7206	Oct. 19, 1909
	Do	9	13.6	80.8	do	7208	Do.
	Do	6	14.3	80.5	do	7214	Do.
	Do	8	15.1	80.6	do	7215	Do.
	Millard						
	Oasis	13	14.2	80.0	do	7929	Oct. 15, 1910
	Do	24	15.8	80.1	do	7940	Oct. 19, 1910
	Do	36	14.1	73.6	do	7971	Oct. 27, 1910
	Do	13	16.8	91.5	do	7996	Oct. 31, 1910
	Do	24	15.8	85.1	do	8006	Nov. 2, 1910
	Do	24	15.6	80.3	do	8019	Nov. 8, 1910
	Do	18	18.2	89.3	do	8053	Nov. 18, 1910
	Hinckley	16	16.6	84.0	do	7954	Oct. 20, 1910
	Do	22	15.3	80.7	do	7955	Do.
	Do	52	14.7	80.7	do	8004	Nov. 2, 1910
	Uinta						
	White Rocks	35	16.0	90.9	Fair	5857	Nov. 2, 1908
	Do	20	15.0	86.2	Good	7904	Oct. 10, 1910
Virginia	Alexandria						
	Arlington	16	19.0	93.1	Fair	6026	Nov. 17, 1908
	Do	16	15.4	79.0	do	6027	Do.
	Do	21	16.0	84.6	do	6045	Nov. 23, 1908
	Do	33	12.0	73.6	do	6046	Do.
	Do	27	10.6	69.8	do	6047	Do.
	Do	23	12.2	75.3	do	6048	Do.
	Do	20	13.9	80.3	do	6049	Do.
	Do	8	10.7	76.9	Good	8037	Nov. 10, 1910
	Augusta						
	Stuarts Draft	16	14.7	87.5	do	5813	Oct. 23, 1908
	Do	12	14.7	83.0	Fair	6016	Nov. 16, 1908
	Do	10	17.0	92.4	do	6017	Nov. 14, 1908
	Do	17	12.5	78.4	Good	7132	Oct. 1, 1909
	Do	11	12.9	79.9	Fair	7133	Do.
	Do	9	14.0	80.2	Good	7211	Oct. 19, 1909
	Do	13	16.0	86.4	do	7212	Do.
	Do	24	13.9	79.7	do	7213	Do.
	Botetourt						
	Troutville	22	12.2	86.5	do	5806	Oct. 22, 1908
	Do	19	14.4	86.7	Fair	5807	Do.
	Do	18	16.3	92.0	Good	5814	Oct. 23, 1908
	Do	20	13.9	81.8	do	6008	Nov. 13, 1908
	Clarke						
	Berryville	15	14.1	85.4	Fair	5932	Nov. 5, 1908
	Do	16	12.6	75.9	Good	7097	Sept. 21, 1909
	Do	16	12.7	78.2	Fair	7098	Do.
	Do	16	13.6	80.4	Good	7099	Do.
	Do	17	10.5	76.6	do	7100	Do.
	Do	12	14.1	76.0	Fair	7127	Sept. 29, 1909
	Do	17	17.0	85.0	Good	7197	Oct. 16, 1909
	Do	18	16.9	82.8	do	7198	Do.
	Do	18	15.0	79.8	do	7226	Oct. 21, 1909
	Do	16	16.3	87.0	do	7227	Do.
	Do	26	15.0	76.1	do	7295	Nov. 1, 1909
	Fauquier						
	Rectortown	15	15.0	88.2	Fair	6001	Nov. 12, 1908
	Loudoun						
	Round Hill	20	13.8	80.7	Good	5805	Oct. 22, 1908
	Bluemont	18	16.0	81.6	Fair	5962	Nov. 10, 1908
	Do	12	15.8	85.4	Good	5988	Do.
	Round Hill	8	14.4	81.3	Fair	5998	Nov. 11, 1908
	Do	20	15.7	87.7	Good	6002	Nov. 12, 1908
	Bluemont	30	15.4	81.8	do	6009	Nov. 13, 1908
	Montgomery						
	Blacksburg	12	18.4	88.9	Fair	6034	Nov. 20, 1908
	Childress	32	5.3	62.0	do	6041	Nov. 21, 1908

Sugar-beet anaylses, made in the Bureau of Chemistry—Continued.

State.	County and town.	Analysis.			Condition of sample.	Serial number.	Date of analysis.
		Average weight.	Sugar in juice.	Purity.			
Virginia	Pulaski □	*Ounces.*	*Percent.*				
	Dublin	32	16.5	86.4	Fair	5781	Oct. 17, 1908
	Do	13	17.5	88.8	do	5782	Do.
	Pulaski	26	14.0	87.5	do	5843	Oct. 30, 1908
	Do	18	15.5	83.7	do	5845	Do.
	Dublin	12	17.5	95.1	Good	5950	Nov. 7, 1908
	Pulaski	10	13.4	84.8	do	7122	Sept. 28, 1909
	Do	18	13.7	85.0	do	7123	Do.
	Do	17	12.5	79.6	do	7125	Do.
	Do	16	14.2	81.8	do	7238	Oct. 23, 1909
	Do	11	16.6	85.8	do	7239	Do.
	Do	16	15.3	84.5	do	7240	Do.
	Do	21	14.0	82.8	do	7241	Do.
	Dublin	16	10.4	71.2	do	7101	Sept. 21, 1909
	Do	24	11.7	76.4	do	7102	Do.
	Do	10	12.0	76.4	Fair	7103	Do.
	Do	14	12.0	78.4	do	7104	Do.
	Do	13	14.2	76.7	do	7242	Oct. 25, 1909
	Do	19	14.6	80.0	Good	7243	Do.
	Do	20	14.6	79.2	do	7244	Do.
	Do	14	12.5	77.1	do	7271	Oct. 27, 1909
	Roanoke □						
	Roanoke	16	14.0	....	Fair—soft	7124	Sept. 28, 1909
	Do	18	11.1	74.3	Good	7143	Oct. 3, 1909
	Do	34	13.4	78.2	do	7230	Oct. 22, 1909
	Rockingham □						
	Grottoes	39	10.5	68.1	Fair	6013	Nov. 14, 1908
	Smyth □						
	Seven Mile Ford	30	14.6	89.2	do	5737	Oct. 10, 1908
	Marion	22	16.6	91.6	do	5738	Do.
	Seven Mile Ford	48	13.9	86.3	do	5844	Oct. 30, 1908
	Atkins	23	16.5	85.1	do	5900	Nov. 4, 1908
	Do	29	16.0	71.1	do	5902	Do.
	Marion	21	16.5	86.3	Good	5945	Nov. 7, 1908
	Do	32	11.8	77.6	do	5947	Do.
	Seven Mile Ford	28	15.9	81.5	do	5989	Nov. 10, 1908
	Do	35	15.4	80.7	Fair	5990	Do.
	Do	24	15.3	79.3	do	5991	Do.
	North Holston	13	15.3	85.7	Good	7121	Sept. 28, 1909
	Do	22	13.6	83.2	do	7126	Do.
	Chilhowie	17	12.5	81.1	Fair	7134	Oct. 1, 1909
	Do	5	12.0	77.2	Good	7135	Do.
	Marion	32	12.5	76.2	do	7136	Do.
	Seven Mile Ford	42	13.4	77.2	do	7138	Oct. 2, 1909
	Do	13	10.7	72.7	do	7139	Do.
	Do	49	8.3	62.7	do	7186	Oct. 12, 1909
	North Holston	10	14.8	81.5	do	7191	Oct. 13, 1909
	Do	12	14.5	80.5	do	7192	Do.
	Marion	40	13.8	81.4	do	7210	Oct. 19, 1909
	Seven Mile Ford	42	14.3	82.5	do	7237	Oct. 23, 1909
	Do	28	11.3	70.6	do	7276	Oct. 28, 1909
	Chilhowie	13	11.3	77.6	do	7965	Oct. 24, 1910
	Do	17	11.3	74.0	do	8045	Nov. 14, 1910
	Southampton □						
	Ivor	17	14.0	79.0	Fair	7105	Sept. 21, 1909
	Tazewell □						
	Burkes Garden	20	13.6	81.4	Good	6005	Nov. 12, 1908
	Tazewell	30	17.1	87.7	do	6023	Nov. 17, 1908
	North Tazewell	8	8.4	60.8	do	7180	Oct. 11, 1909
	Warren □						
	Linden	20	13.7	79.1	do	6000	Nov. 11, 1908
	Do	36	15.5	83.7	do	6029	Nov. 17, 1908
	Washington □						
	Abingdon	15	16.9	85.3	do	5928	Nov. 5, 1908
	Do	15	16.8	89.8	do	5929	Do.
	Do	15	16.4	87.2	do	5930	Do.
	Konnarock	24	13.2	81.9	do	7116	Sept. 27, 1909
	Abingdon	17	14.5	78.8	do	7140	Oct. 2, 1909
	Glade Spring	10	14.9	82.7	do	7141	Do.
	Konnarock	18	15.0	83.3	do	7189	Oct. 13, 1909
	Do	34	13.0	81.2	do	7190	Do.
	Abingdon	19	14.8	82.6	do	7194	Oct. 14, 1909
	Do	24	11.1	76.0	do	7930	Oct. 17, 1910
	Do	36	13.0	81.9	do	7956	Oct. 20, 1910
	Do	24	14.4	82.9	do	8005	Nov. 2, 1910

Sugar-beet analyses, made in the Bureau of Chemistry—Continued.

State.	County and town.	Analysis.			Condition of sample.	Serial number.	Date of analysis.
		Average weight.	Sugar in juice.	Purity.			
Virginia	Wythe	*Ounces.*	*Per cent.*				
	Rural Retreat	16	13.8	78.8	Good	4883	Oct. 14, 1907
	Do	16	17.0	83.7		5154	Nov. 20, 1907
	Do	10	14.6	88.0	Fair	5996	Nov. 11, 1908
	Do	11	14.7	89.1	do	5997	Do.
	Do	34	17.7	84.6	Good	6032	Nov. 19, 1908
	Do	31	16.3	84.0	do	6044	Nov. 23, 1908
	Wytheville	8	19.0	82.5	Soft	7423	Nov. 23, 1909
Washington	Kitsap						
	Manette	9	19.2	89.0	Wilted	8157	Dec. 10, 1910
West Virginia	Jefferson						
	Shenandoah Junction.	16	12.3	70.1	Good		
	Charlestown	16	12.2	74.8	do	7928	Oct. 15, 1910
	Do	48	13.6	79.5	do	7957	Oct. 21, 1910
	Do	16	12.6	71.3	do	7958	Do.
	Do	32	15.0	82.6	do	7960	Do.
	Do	20	12.7	77.4	do	8018	Nov. 5, 1910
	Shenandoah Junction.	20	16.2	78.8	do	7968	Oct. 26, 1910
	Do	16	13.8	80.0	do	7972	Oct. 27, 1910
	Do	20	20.3	78.2	Bad	8003	Nov. 2, 1910
	Do	36	14.6	80.4	Good	8010	Nov. 3, 1910
	Kearneysville	20	13.2	81.1	do	7964	Oct. 24, 1910
	Do	16	14.7	77.3	do	8038	Nov. 10, 1910
	Do	6	17.7	84.0	do	8092	Nov. 23, 1910
	Summit Point	10	7.3	59.5	do	8021	Nov. 8, 1910
	Preston						
	Reedsville	21	18.3	86.9	do	8039	Nov. 10, 1910
	Roane						
	Bloomington	20	6.9	63.0	do	8001	Nov. 1, 1910
	Summers						
	Lowell	32	11.8	79.3	do	7993	Oct. 29, 1910
Wyoming	Carbon						
	Baggs	22	14.8	79.4	do	7892	Oct. 18, 1910
	Crook						
	Forest	32	11.5	74.2	do	7274	Oct. 28, 1909
	Laramie						
	Cheyenne	23	12.8	73.4		4886	Oct. 15, 1907
	Do	11	19.3	82.8		4891	Oct. 23, 1907
	Chugwater	25	15.9	80.9	Red—small	5213	Dec. 3, 1907
	Do	10	6.4	55.1	do	5225	Do.
	Cheyenne	18	16.0	81.6	Good	5705	Oct. 6, 1908
	Do	16	24.3	92.0	Fair	5773	Oct. 15, 1908
	Sheridan						
	Kendrick	14	9.5	68.8	Poor	5804	Oct. 22, 1908
	Weston						
	Cambria	8	17.8	86.2	Fair	7137	Oct. 2, 1909
	Horton	14	11.2	79.0	do	7939	Oct. 18, 1910
	Uinta						
	Lyman	26	15.7	83.1	Good	5780	Oct. 17, 1908

YEARLY AVERAGE ANALYTICAL DATA BY STATES, FROM 1884 TO 1900, AND FROM 1905 TO 1910.

The following table contains the yearly average analytical figures of the bureau for beets grown in the various States since 1884, with the exception of the years 1901–1904. The figures are of value in showing the variability of the sugar content and purity by years and in providing a basis for a comparison of the various States as sugar-beet producers. Unlike the preceding table, the sugar content has been figured by a factor to the sugar in the beet. The results for the years up to 1898 are taken from Bulletin No. 52 of the Bureau of Chemistry, while the results for 1898, 1899, and 1900 are taken from the Report of Progress of Beet Sugar Industry in the United States for these years. The results for 1905 to 1910 are based on the figures given in this report.

Yearly average of analyses of beets, by States, made in Bureau of Chemistry, 1884 to 1900 and 1905 to 1910.

State and year.	Number of samples.	Average weight.	Sugar in beet.[1]	Purity.
Alabama:		*Ounces.*	*P. ct.*	
1893	......	......	5.9	66.7
1898	4	18	8.5	73.2
1909	1	13	12.4	77.6
Arizona:				
1891	2	51	7.7	56.9
1897	7	23	9.3	70.4
1908	1	16	13.9	77.2
Arkansas:				
1891	2	40	6.4	58.8
1892	3	12	9.4	64.7
1897	2	18	11.3	71.5
1898	6	23	7.1	67.5
1899	5	15	6.0	55.5
1900	2	9	6.7	61.6
1906	1	8	10.8	80.3
1907	1	5	14.4	78.8
1909	1	16	12.3	......
1910	4	16	10.9	75.4
California:				
1884	71	19	13.7	85.3
1890	4	13	14.7	84.6
1891	8	48	11.1	75.8
1892	4	14	14.7	77.6
1897	1	26	16.8	......
1898	4	25	14.6	80.2
1899	1	11	13.9	82.0
1900	4	13	12.9	78.9
1908	6	13	13.3	82.2
1909	7	23	13.5	79.9
1910	2	32	16.8	82.5
Colorado:				
1890	29	20	12.5	76.1
1891	51	26	13.1	76.1
1892	170	18	14.8	81.7
1893	18	17	13.2	74.9
1897	174	20	13.6	76.7
1898	50	22	13.7	80.1
1899	64	24	14.4	80.2
1900	57	25	14.1	78.7
1905	22	29	16.1	82.6
1907	2	19	19.7	86.7
1908	9	17	13.6	79.5
1909	24	35	16.7	82.8
1910	4	20	11.9	70.9
Connecticut:				
1890	2	14	9.7	76.1
1891	5	27	10.8	77.3
1898	4	21	10.3	76.2
1899	2	17	10.9	75.5
1900	1	20	10.0	73.4
Delaware.				
1898	1	14	11.3	78.8
1899	2	24	12.5	81.4
1900	1	15	10.0	75.6
Georgia:				
1891	2	12	11.1	64.9
1898	4	47	5.8	64.0
1899	10	14	11.0	75.0
1900	1	10	14.2	86.6
1908	2	33	9.6	73.7
1909	1	14	8.5	71.6
1910	2	11	9.6	73.2
Idaho:				
1890	1	4	8.0	68.3
1891	1	15	12.7	74.9
1892	2	34	14.7	79.1
1893	2	78	10.2	76.2
1897	7	21	15.5	79.4
1898	5	28	12.0	78.3
Idaho—Continued.		*Ounces.*	*P. ct.*	
1899	1	36	10.8	75.0
1900	19	26	13.5	81.4
1906	1	24	19.1	89.5
1910	2	27	16.3	83.5
Illinois:				
1890	8	31	10.3	72.1
1891	36	32	11.7	76.4
1892	59	15	10.9	75.2
1897	32	17	13.1	75.5
1898	38	20	10.2	75.2
1899	25	25	10.6	72.6
1900	16	27	8.3	65.2
1905	7	20	11.6	76.1
1906	27	19	16.0	81.6
1908	1	12	18.8	88.0
1909	7	29	12.0	76.2
1910	3	41	9.6	71.5
Indiana:				
1890	56	23	10.7	72.7
1891	77	27	11.6	76.9
1892	57	14	11.2	72.5
1893	4	10	10.7	73.1
1897	103	14	13.1	78.9
1898	88	21	10.1	75.5
1899	29	19	11.4	73.4
1900	15	21	9.4	71.1
1909	3	26	11.6	75.6
1910	2	42	11.1	74.8
Indian Territory:				
1891	1	27	11.6	75.9
1898	1	27	9.6	77.1
1900	3	29	7.9	66.2
Iowa:				
1890	30	22	11.8	74.5
1891	321	30	11.8	75.7
1892	30	24	10.9	76.2
1893	7	17	12.8	75.8
1897	130	18	13.3	73.7
1898	147	25	11.4	76.1
1899	67	24	10.9	72.1
1900	39	33	9.5	70.7
1905	16	24	11.3	76.1
1908	2	28	14.8	87.7
1909	10	30	11.3	75.2
Kansas:				
1890	22	32	8.3	69.3
1891	36	33	10.7	68.2
1892	22	25	11.1	74.2
1893	1	......	14.3	72.8
1897	41	27	11.4	73.8
1898	16	22	10.3	71.3
1899	35	22	9.6	66.0
1900	20	25	10.1	72.1
1907	11	19	10.8	72.3
1908	6	28	11.4	75.2
1909	1	24	15.3	82.1
1910	3	7	15.7	78.5
Kentucky:				
1891	3	34	9.1	63.7
1892	4	13	8.9	77.2
1897	6	16	11.9	71.5
1898	4	14	5.9	61.1
1899	1	5	7.4	......
1900	12	13	8.5	64.8
Louisiana:				
1893	3	12	10.3	72.2
1910	1	5	9.0	70.8
Maine:				
1900	2	19	8.1	67.7

[1] Calculated from sugar in juice by factor.

Yearly average of analyses of beets, by States, made in Bureau of Chemistry., 1884 to 1900 and 1905 to 1910—Continued.

State and year.	Number of samples.	Average weight.	Sugar in beet.	Purity.
Maryland:		*Ounces.*	*P. ct.*	
1890	83	15	12.2	79.3
1891	2	16	7.4	68.5
1897	29	19	11.4	79.1
1898	31	22	10.4	76.0
1899	6	18	10.2	74.6
1900	1	10	9.3	74.2
1905	1	21	16.6	79.8
1910	9	15	14.5	84.0
Massachusetts:				
1890	6	16	12.0	82.8
1898	4	27	12.0	78.6
1899	9	21	14.6	83.3
1900	2	8	14.0	......
Michigan:				
1890	30	31	12.0	78.4
1891	50	32	12.6	78.0
1892	71	19	14.1	83.4
1893	88	15	13.3	82.1
1897	450	22	14.7	81.1
1898	34	28	13.2	81.9
1899	236	22	13.1	79.7
1900	478	14	11.3	76.7
1905	21	15	16.4	82.8
1906	28	22	15.9	83.5
1907	2	18	15.6	86.1
1909	2	17	16.0	86.0
Minnesota:				
1890	107	30	11.8	75.2
1891	41	29	12.4	75.7
1892	22	29	12.2	78.1
1893	7	60	10.8	70.8
1897	49	24	11.0	79.2
1898	21	22	12.7	78.7
1899	9	23	12.3	77.5
1900	10	31	10.9	75.9
1910	1	9	9.3	66.6
Mississippi:				
1898	2	20	4.2	65.7
1908	1	....	12.4	78.3
1909	5	13	9.9	69.9
1910	1	20	9.2	72.7
Missouri:				
1890	2	21	8.4	66.7
1891	67	20	10.4	62.4
1892	13	33	8.1	63.4
1897	324	20	11.7	73.5
1898	43	17	8.5	68.6
1899	19	17	7.1	64.3
1900	9	15	8.5	67.2
1908	3	13	12.2	79.7
1909	5	16	10.5	72.4
1910	4	10	8.7	69.9
Montana:				
1891	35	25	13.2	76.8
1892	6	22	10.9	72.8
1893	2	15	14.3	75.0
1897	4	20	14.4	77.8
1898	7	21	11.2	72.6
1899	2	40	10.7	70.6
1900	4	38	10.4	69.3
1909	1	24	16.0	76.8
1910	2	14	15.9	86.1
Nebraska:				
1890	269	20	11.8	71.9
1891	62	35	11.7	75.3
1892	27	21	14.2	79.3
1893	8	17	10.1	69.7
1897	13	29	12.9	76.9
1898	10	25	12.8	76.8
1899	6	19	11.3	74.4
1900	11	28	9.9	72.1
1908	5	19	9.1	68.3
1909	4	23	15.0	80.8
Nevada:				
1891	18	11	17.2	88.0
1892	81	13	15.9	83.4
1897	21	18	18.3	81.4
1898	42	12	18.5	85.9
1899	24	18	16.9	82.2
1900	3	10	9.6	79.6
Nevada—Contd.		*Ounces.*	*P. ct.*	
1908	5	22	16.0	85.8
1909	13	23	17.5	87.4
New Hampshire:				
1891	1	19	11.6	80.0
1898	2	34	13.5	83.5
1899	4	17	15.5	86.0
1900	9	29	11.1	74.9
New Jersey:				
1891	1	17	7.3	70.8
1897	31	16	14.2	81.4
1898	33	20	11.1	77.5
1899	17	27	11.3	77.3
1900	2	21	11.4	76.7
1909	1	16	7.9	63.5
New Mexico:				
1891	17	28	13.8	74.8
1892	29	19	15.3	83.2
1897	3	13	17.2	82.0
1898	7	20	12.8	78.0
1899	2	22	14.9	82.9
1900	2	23	14.5	77.8
1906	2	20	15.3	78.1
1907	20	21	17.9	83.2
1908	51	19	15.4	84.7
1909	14	19	14.6	80.1
1910	1	17	16.0	77.0
New York:				
1890	10	15	12.1	78.0
1891	4	32	11.6	76.8
1892	8	22	15.4	85.9
1897	225	21	15.0	82.4
1898	328	21	12.6	80.5
1899	142	19	13.0	78.8
1900	51	22	13.3	79.8
1910	2	10	13.8	83.5
North Carolina:				
1892	4	4	9.0	73.4
1897	7	23	9.1	75.3
1898	14	19	6.5	61.8
1899	2	17	7.6	69.0
1900	4	23	10.3	76.4
1907	7	17	12.2	80.8
1908	2	26	9.3	72.6
1909	4	14	14.0	82.6
1910	1	24	13.5	82.7
North Dakota:				
1890	24	25	13.4	71.2
1891	11	23	11.8	73.2
1892	11	24	12.9	76.5
1893	2	27	14.0	80.7
1897	4	28	10.5	81.2
1899	3	22	13.9	78.3
1900	5	22	10.2	71.1
1905	6	35	12.7	73.9
1908	7	15	14.4	83.6
1910	4	24	13.9	76.9
Ohio:				
1890	15	26	9.8	76.0
1891	66	31	11.3	73.5
1892	102	17	14.2	80.2
1897	68	22	13.8	79.1
1898	409	24	11.0	77.1
1899	128	24	11.9	76.1
1900	64	26	10.3	73.1
1906	5	27	17.0	81.7
1907	15	14	18.1	87.8
1908	1	16	9.5	73.0
1909	2	18	15.6	85.2
1910	2	19	11.9	75.0
Oklahoma:				
1891	1	48	6.4	53.3
1897	1	10	11.8	72.5
1898	6	24	10.2	73.3
1899	2	31	10.3	69.3
1900	4	22	10.6	69.8
1907	3	23	14.7	78.1
1908	7	25	13.8	81.2
1909	16	15	12.0	74.5
1910	4	19	13.4	81.9

Yearly average of analyses of beets, by States, made in Bureau of Chemistry, 1884 to 1900 and 1905 to 1910—Continued.

State and year.	Number of samples.	Average weight.	Sugar in beet.	Purity.
Oregon:		*Ounces.*	*P. ct.*	
1890	2	20	15.1	73.4
1891	35	34	12.7	81.1
1892	12	19	14.2	80.2
1898	6	20	14.1	83.4
1899	1	17	15.8	84.3
1900	11	19	12.4	78.9
1905	2	16	14.3	81.0
1906	1	32	12.2	68.0
1907	3	26	17.1	83.1
1908	3	17	17.0	86.2
1910	15	21	15.6	81.3
Pennsylvania:				
1890	10	27	8.0	73.8
1891	7	22	13.3	78.7
1892	8	13	10.8	75.8
1893	1	....	11.0	78.9
1897	59	18	13.8	79.5
1898	81	21	11.6	78.1
1899	28	31	11.2	75.4
1900	11	21	10.6	74.8
1907	1	13	15.3	79.7
1908	3	18	14.2	82.4
1909	1	12	14.2	81.4
Rhode Island:				
1897	2	21	11.9	74.2
South Carolina:				
1897	13	17	9.9	79.9
1898	4	14	10.2	81.2
1899	4	14	13.0	79.3
1900	3	29	8.4	69.5
South Dakota:				
1890	21	20	13.1	78.6
1891	202	22	12.5	75.3
1892	67	20	13.1	75.5
1897	5	17	15.1	83.2
1898	24	16	13.9	78.6
1899	11	25	10.6	72.8
1900	9	29	10.5	71.7
1907	1	16	17.1	75.3
1909	3	13	16.6	82.8
Tennessee:				
1891	5	20	8.8	65.8
1892	1	10	9.4	72.4
1897	17	11	10.8	71.9
1898	10	17	8.0	69.3
1899	2	54	8.3	67.6
1900	1	15	5.4	54.8
1909	14	15	9.7	71.1
1910	2	25	10.3	72.8
Texas:				
1890	2	38	10.0	69.3
1891	10	23	10.3	69.1
1897	11	22	12.6	76.5
1898	49	25	9.5	69.8
1899	3	12	7.5	53.7
1900	15	29	6.9	56.8
1905	24	27	12.5	75.6
1906	17	27	13.2	75.8
1907	8	19	14.3	73.3
1908	24	20	12.7	77.6
1909	26	15	15.1	77.6
1910	10	20	13.1	76.9
Utah:		*Ounces.*	*P. ct.*	
1897	35	20	14.3	81.1
1898	14	16	13.6	85.3
1899	10	20	15.0	83.6
1900	5	14	13.8	81.3
1908	1	35	15.2	90.9
1909	4	8	13.2	79.4
1910	11	23	14.9	82.7
Vermont:				
1897	8	22	14.2	84.1
1898	68	22	13.2	82.8
1899	16	23	12.8	79.0
1900	3	15	12.1	79.7
Virginia:				
1890	20	15	10.8	74.0
1891	72	21	11.1	76.0
1892	13	12	12.0	79.6
1893	14	16	13.3	83.9
1897	34	21	11.6	76.2
1898	43	20	8.9	72.4
1899	6	17	9.5	74.2
1900	49	18	10.0	74.1
1907	2	16	14.7	81.2
1908	55	21	14.4	83.7
1909	51	19	12.9	79.1
1910	6	20	11.3	78.2
Washington:				
1890	1	16	15.2	84.2
1891	11	18	14.5	83.9
1892	31	18	14.5	76.8
1893	183	28	12.3	74.0
1897	34	27	13.7	80.7
1898	5	27	13.9	81.3
1899	8	23	13.0	77.8
1900	2	24	14.9	85.6
1910	1	9	18.3	89.0
West Virginia:				
1892	12	14	11.3	68.5
1897	14	19	15.4	80.4
1898	4	28	9.1	72.9
1899	3	20	9.1	67.2
1900	4	7	10.7	62.7
1909	1	16	11.7	70.1
1910	16	22	13.2	77.1
Wisconsin:				
1890	10	21	12.8	81.3
1891	432	26	11.1	75.8
1892	21	22	12.7	77.8
1897	42	15	15.8	83.3
1898	16	24	13.0	79.3
1899	25	21	14.8	84.4
1900	18	30	10.0	73.1
Wyoming:				
1890	5	26	15.1	78.8
1891	18	12	13.5	78.1
1892	6	8	15.2	85.2
1893	48	19	15.9	80.5
1897	34	19	17.2	82.3
1898	10	19	13.9	78.1
1899	1	29	15.9	81.9
1900	2	20	13.5	78.5
1907	4	17	13.0	73.0
1908	4	19	15.5	81.4
1909	2	20	14.0	80 1
1910	2	18	12.4	79.2

BIBLIOGRAPHY OF CHEMICAL LITERATURE ON METHODS OF ANALYZING BEETS (1839 TO 1906, INC.).

This bibliography does not pretend to be complete, but it contains titles of papers that have been noted by the author in a search through the complete files of the Pharmaceutische Centralblatt, Chemisches Centralblatt, Zeitschrift des Vereins der Rubenzuckerindustrie and Zeitschrift des Vereins der deutschen Zuckerindustrie, Scheibler's Neue Zeitschrift der Rubenzuckerindustrie, Zeitschrift für angewandte Chemie, La Sucrerie Indigene, and of certain volumes of the Bulletin de l'Association des Chimistes de Sucrerie et de Distillerie, Die deutschen Zuckerindustrie, Osterreichische-Ungarische Zeitschrift für Zuckerindustrie und Landwirtschaft, and Zeitschrift für Zuckerindustrie in Böhmen.

After having completed this review, a volume by E. O. Von Lippmann on "Die Entwicklung der deutschen Zuckerindustrie von 1850 bis 1900" was found in which the author devotes some 10 pages to abstracts, with references, of articles on analysis and valuation of sugar beets. The papers referred to there, with few exceptions, are noted in this bibliography. The list is made by years, with the authors' names in alphabetical order. It has been brought up to 1907, as from that time on such literature will be found in the volumes of Chemical Abstracts, American Chemical Society.

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H. W. WILEY, CHIEF OF BUREAU.

COAL-TAR COLORS USED IN FOOD PRODUCTS.

BY

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LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Chemistry,
Washington, D. C., January 31, 1911.

Sir: I have the honor to transmit for your approval a report by Bernhard C. Hesse, a color expert, containing both original chemical work done in the bureau since the passage of the food law on anilin dyes used for foods, and a valuable and extensive compilation of the literature of the subject, especially with reference to the harmfulness of coal-tar colors and their physiological effects. These data formed the basis of the opinions stated in Food Inspection Decisions 76, 77, and 106, and are presented in detail as of scientific and practical interest to all those concerned in the use of coal-tar colors in foods, whether as manufacturers, food officials, or consumers. I recommend the publication of this report as Bulletin No. 147 of the Bureau of Chemistry.

Respectfully,
H. W. Wiley, *Chief.*

Hon. James Wilson,
Secretary of Agriculture.

CONTENTS.

Page.

Introduction 9
- Purpose of the investigation 9
- Number of colors permitted 11
- Quality and efficiency of colors permitted 12

I. Identity of coal-tar colors used in food products in the United States in 1907 15
- Collection of samples 15
- Classification of samples submitted 17
  - Green Table numbers 17
  - Source 18
  - Patents 18
  - Shades of color 19

II. Purposes of food coloring 23

III. Food color requirements 25
- Adaptability for special purposes 25
- Proportion of coal-tar color used 26
- Suitability of shades of permitted colors and mixtures of same 28

IV. Conformity of food color market, 1907, to recommendations of the National Confectioners' Association, 1899 30

V. Some legal enactments relative to the use of coal-tar dyes 35
- List of thirteen foreign legal enactments 35
- Summary of colors permitted by these legal enactments 35
- Colors said to be permitted under the German law of 1887 37
- Definiteness and detail necessary to effect quality control 40
- State laws prohibiting the use of colors in certain foods, 1909 41

VI. Recommendations by associations and individuals as to use of coal-tar dyes as food colors 42
- Cazeneuve and Lépine 42
- Society of Swiss Analytical Chemists 43
- Tschirch 43
- Kayser 44
- Weyl 44
- National Confectioners' Association 45
- Schacherl 45
- Classification of recommendations in the literature 46
- Conclusions 47

VII. Recommendations made by United States color industries and trades to the Department of Agriculture 47
- Antagonistic to all added artificial color 48
- Concerning restrictions and requirements 48

VIII. Investigations, other than on animals, bearing on the harmfulness of coal-tar colors 51
- Pfeffer 51
- Winogradow 52
- Heidenhain 52
- Other authors 54

Page.
IX. Compilation under the Green Table numbers of all information available as to the suitability of coal-tar colors for food 56
  General statements 56
  Classification of opinions in literature and in legal enactments showing condition of the market in 1907 62
  Classification according to chemical composition and suitability 64
  Physiological action of coal-tar dyes 67
    Summary of symptoms 67
      Experiments on dogs 67
      Experiments on human beings 70
      Experiments on small animals 71
    General statements 71
  Complete detailed statement of all combined data 74
    Abbreviations of authorities cited 74
    Tabulation by Green Table numbers of physiological and other data 75
    Alphabetical index of trade names of coal-tar colors 148
X. Dosage and symptoms 153
  Confectioner's list as a basis for a rule 153
  Lehmann's rules 158
  Santori's work as a guide to a rule 158
  Young's rule 159
XI. Oil-soluble or fat colors 159
XII. Rules and reasons for selecting the seven colors permitted by F. I. D. 76 161
  Statement of rules 161
  Analysis of three recommendations made to the Department of Agriculture 162
  Process of elimination 166
  Reasons for adding Ponceau 3R 167
  Quality, cleanliness, and efficiency 169
XIII. Lists of colors subsequently recommended by individuals and associations 169
  Ernst 170
  Muttelet's interpretation of the French law 171
  Second International White Cross Congress 172
  Beythien and Hempel 173
  Summary of three preceding recommendations 174
  Unpublished recommendations of a manufacturer 177
  Béhal 177
  Conclusions 178
XIV Chemical examination of the seven permitted colors, 1907 179
  Need of chemical control 179
  First methods of analysis used 181
    Moisture 182
    Chlorin as chlorids 182
    Sulphated ash 182
    Total sulphur 183
    Gutzeit test 183
    Heavy metals 183
    Total insolubles 184
    Ether extractive 184
  Results of chemical examination, 1907 184
    Detailed chemical data on each permitted color 184
    Recalculation of analytical data on basis of coloring matter present 187
  Market quality of the seven permitted colors 190

Page.
XV. Guides in determining degree of purity and cleanliness 192
XVI. Analyses of certified lots of permitted colors, 1909–10 198
Tabulation of results 198
Comparison of analyses made in 1907 201
Conformity of analytical data with theoretical composition 202
Arsenic determinations on 86 batches 204
Suggested requirements for certified colors 205
XVII. Methods of analysis used in testing colors for certification 210
Introduction 210
Naphthol Yellow S 211
Ponceau 3R 215
Orange I 217
Amaranth 220
Light Green S F Yellowish 221
Erythrosin 222
Indigo disulphoacid 224
XVIII. Addenda 225
Additional examination of coal-tar dyes 225
Supplementary list of trade names of coal-tar colors 227
XIX. Index of authorities quoted 227

ILLUSTRATION.

Page.
FIG. 1. Apparatus used in the determination of arsenic 213

COAL-TAR COLORS USED IN FOOD PRODUCTS.

INTRODUCTION.

PURPOSE OF THE INVESTIGATION.

For the purposes of the investigation reported in the following pages, the legitimacy of the coloring of food and food products under certain conditions is regarded as established; the ethical and dietetic aspects of the question of food coloring are not here considered.

The means at hand for coloring food products may be conveniently classified as vegetable, animal, mineral or inorganic, and synthetic or so-called coal-tar colors or dyes. Representatives of each of these have at one time or another all been used in the coloring of food, and the laws of various European and American States have, from time to time prohibited the use of certain specified members or all of each or some of the foregoing classes. It is therefore obvious that even for the legitimate purposes for which food can be colored, improper means are at command, and some of these, if not all, have been prohibited by law at some time or another.

It is the function of the present work to determine what members of the synthetic or coal-car colors should be considered legitimate for coloring foods. It is confidently believed that the material collected in the following pages points clearly and solely to the following conclusions:

1. Coal-tar dyes should not be used indiscriminately in foods.
2. Only specified coal-tar dyes should be used in foods.
3. Only tested and certified dyes should be used in foods.

The work here reported has furnished the basis for Food Inspection Decisions Nos. 76, 77, and 106, issued July 13, 1907, September 25, 1907, and March 25, 1909, respectively, and the investigation itself was practically terminated January 1, 1910.

The effect of these decisions has been to restrict the coal-tar colors permitted for use in foods to seven specified and enumerated colors, until such time as it shall be shown with reasonable conclusiveness that other colors should be added to such list; and further, all coal-tar colors permitted for use in food are to be of a degree of purity and cleanliness acceptable to the Department of Agriculture, and are to be so certified.

In order to avoid any uncertainty as to the chemical composition of the enumerated colors, direct reference is made in Food Inspection Decision No. 76 to a standard work in which such chemical composition is clearly and unequivocally set forth. The relevant parts of Food Inspection Decision No. 76 are as follows:

> The use of any dye, harmless or otherwise, to color or stain a food in a manner whereby damage or inferiority is concealed is specifically prohibited by law. The use in food for any purpose of any mineral dye or any coal-tar dye, except those coal-tar dyes hereinafter listed, will be grounds for prosecution. Pending further investigations now under way and the announcement thereof, the coal-tar dyes hereinafter named, made specifically for use in foods, and which bear a guaranty from the manufacturer that they are free from subsidiary products and represent the actual substance the name of which they bear, may be used in foods. In every case a certificate that the dye in question has been tested by competent experts and found to be free from harmful constituents must be filed with the Secretary of Agriculture and approved by him.
>
> The following coal-tar dyes which may be used in this manner are given numbers, the numbers preceding the names referring to the number of the dye in question as listed in A. G. Green's edition of the Schultz-Julius Systematic Survey of the Organic Coloring Matters, published in 1904.
>
> The list is as follows:
>
> *Red shades:* 107. Amaranth. 56. Ponceau 3 R. 517. Erythrosin.
> *Orange shade:* 85. Orange I.
> *Yellow shade:* 4. Naphthol Yellow S.
> *Green shade:* 435. Light Green S F Yellowish.
> *Blue shade:* 692. Indigo disulfoacid.
>
> Each of these colors shall be free from any coloring matter other than the one specified and shall not contain any contamination due to imperfect or incomplete manufacture.

The reasons, broadly considered, which led up to these food inspection decisions are given in concise fashion in this introduction.

Looking over the restrictions placed upon coal-tar colors by the lawmakers of the various countries it will be found that certain colors are in some instances specifically prohibited and in other instances that certain specific colors, or classes of colors, and only such, are permitted for the legitimate purposes of food coloring.

Private organizations, such as the Swiss Society of Analytical Chemists and the National Confectioners' Association in the United States, have also made recommendations permitting specific colors only, and in addition specifically prohibiting others. Individual authors have likewise made similar recommendations. The control of the quality of the food colors practiced on the part of those Governments which restrict the use of coal-tar colors to certain individuals, so far as any publications show, has not been very extensive.

The action taken against the use of coal-tar colors for food-coloring purposes has ranged all the way from absolute prohibition of their use for any purpose whatsoever to the practically unlimited use in legitimate food coloring operations of all but two of such colors.

Intermediate between these two extremes we find the prohibition of a greater number than two, or of all the members of this class except certain specified colors, and even here with the restriction that they shall be used only for certain legitimate food-coloring purposes.

It would be desirable to have a number of coal-tar colors of established harmlessness specifically permitted, particularly if the number be sufficient to meet all the legitimate demands arising in the food-coloring art. To prohibit only specified coal-tar colors and, by implication, to permit all the rest of this class, would allow the unrestricted use of newly discovered colors, and all other coal-tar colors not examined as to their effect on health. A limited list of permitted coal-tar colors which would make the use of all coal-tar colors outside of the permitted list illegal would properly protect the health and could work no substantial hardship upon those engaged in food coloring. Any such hardship would be avoided by providing that if it is shown that none of the colors of the permitted list meets certain legitimate requirements and that coal-tar colors outside the permitted list are capable of satisfying this need and are in and of themselves harmless the permitted list can be expanded by the proper authorities to meet additional needs or growing requirements without exposing the public health to any risk.

NUMBER OF COLORS PERMITTED.

It will be shown in the following pages that in the summer of 1907 there were on the market of the United States 80 different chemical individuals, or so-called coal-tar colors, offered for the coloring of food. It has been known since 1888 that it is unsafe to attempt to predict the harmfulness or the harmlessness of coal-tar colors by inference or analogy; therefore an ideally perfect permitted list should contain only such colors as have each been examined physiologically, separately, and specifically, and their harmlessness determined by actual test. Out of the 80 colors referred to 30 had not been examined at all, so far as the literature shows, and therefore their harmlessness is certainly open to question; 26 had been examined physiologically, and the published accounts with respect to their harmlessness or their harmfulness are in each case contradictory; on 8 none but adverse reports were to be found in literature, leaving only 16 out of 80 colors on the market which had been established with more or less certainty as harmless—that is, the users of these 8 colors were deliberately taking chances with the public health, since the harmful nature of those 8 had for a long time past been known to those conversant with such subjects; the use of the 26 doubtful colors is more defensible than the use of the 8 known to be harmful. Out of the 30 of whose action nothing was known it can not be said how many are or are not harmful, nor can the risk forced upon the public health be satisfactorily measured.

This brief summary must suffice for the present as a justification for the restrictions of the permitted colors to 7 in number. The full reasons for each and every step will appear in their proper places in the pages following.

QUALITY AND EFFICIENCY OF COLORS PERMITTED.

An examination of 30 specimens representative of the 7 selected permitted colors on the United States market in the summer of 1907 disclosed such a condition of uncleanliness of product, or careless or improper manufacture, and the use of such utterly inferior qualities of products for food coloring purposes, that control over the quality of the seven permitted colors seemed necessary. The results of the work in the making and maintaining of standards of quality for each of these seven colors also justify this control. That there was in 1907, and for a year or more later, a considerable divergence of opinion among chemists as to what should be the proper quality requirements for these colors is shown by the fact that out of 72 foundation certificates offered in accordance with Food Inspection Decisions Nos. 76 and 77, 57 were rejected on their face because they did not comply with the standards of quality then in mind, or then shown to be commercially attainable. Much objection has been made by many of those whose certificates were rejected on the ground that the standards then in mind were unreasonable, unjustifiable, and nonattainable. The actual results, however, are that with very few exceptions the standards in mind early in the work have all been exceeded in practice; the 41,000 pounds (20.5 tons) of certified colors now in existence made in 97 batches, or an average of more than 420 pounds per batch, are, with the exception of perhaps one or two first batches, far cleaner than was expected when the 57 certificates above referred to were rejected.

There has been no complaint against the permitted colors for want of efficiency or for the possession of unsuitable attributes, which has been pressed or sustained with any such earnestness as would reasonably be expected if the defects complained of were as great as they were represented. Complaints have been made against the yellow, when used in acidulated fruit sirups, on account of its possessing a bitter taste; the proof of this, so far as any has been offered, was for a long time not of a convincing nature, and it was two years after the first objection was raised before any concerted or positive action was taken by those interested. The yellow has also been criticized because it is not sufficiently fast to light; although it was satisfactorily shown that another yellow was faster to light than the permitted yellow, no one has maintained that the yellow desired was actually preferable to the permitted yellow wholly and solely because of its superior fastness to light.

The blue has been criticized because it is not of the proper shade to permit of its use in the bluing of sugar, but the substitute offered therefor has not been supported by its sponsors in a way to indicate that a defect of serious magnitude exists. On the grounds of sufficiency and of efficiency the list of permitted colors selected appears to have been justified by the absence of any real or substantial complaint against them, on either or both of these grounds, during a period of more than three years.

None of the seven permitted colors is patented; their manufacture and their purification are open to all, and none of the 80 colors on the market in the summer of 1907, with perhaps one exception, had been discovered since 1891; in other words, the advances in the coal-tar industry from 1891 to 1907 had added nothing to the colors serviceable to the art of food coloring.

The list of colors permitted in Food Inspection Decision No. 76 embraces, therefore, a sufficient number of colors for all legitimate food-coloring purposes, the coloring of fats, oils, butter, etc., excepted, for which no suitable color had been examined and reported in the literature as being harmless and fit for use in foods; they can be made by any one; no one can have a monopoly in any one of them by virtue of patents; any competent maker can make all or any of them and purify them to the required degree of cleanliness. The standards growing out of the control exercised by the Department of Agriculture are such as to insure that the colors used for food-coloring purposes possess a proper degree of cleanliness and such a degree of cleanliness is commercially feasible and is a commercial reality.

The policy adopted in this respect is therefore justified not only from the viewpoint of the history of the attempts on the part of various governments to control the quality of food colors, but also by the results actually obtained by its adoption. This policy of restricting food colors to certain chemical individuals and demanding that those possess certain qualities is in complete harmony with the following suggestion made to the commission on rules and regulations under the food and drugs act, at its hearing in New York, in September, 1906.

> Any kind of a harmless color should be permitted, provided it is not a color generally known to be poisonous, or generally found to be poisonous, or one that may be almost impossible to be produced without containing some poison within itself when finished and ready for use. Coal-tar colors, as a class, should not be prohibited, but all those coal-tar colors generally found to be poisonous, or which are hard to produce without containing some poisonous properties when ready for use, should be forbidden the privilege of being used, or offered for sale for use in food.
>
> Under the provisions of section 2, we have this to recommend to the commission, that every person selling or using a coal-tar color in food or drink, should be required

> to secure, either on his own account, or from the person from whom he buys such color, a certificate to the effect that the identical color used has been tested for poisonous ingredients, and is, to the knowledge of the chemist making the test, absolutely harmless. The chemist should be required to be a competent physiological chemist, and must certify as above under oath. This would mean not that each package of color would have to be tested, but that every batch would have to be tested, and the certificate would then be held to relate to every batch. Such tests should be made in the United States and the chemist certifying should reside in and be a citizen of the United States.
>
> It should not be deemed sufficient to have any particular brand of coal-tar color tested once, and a blanket certificate given, covering the whole brand as long as it may be sold, but every ounce of coal-tar color put out by a color manufacturer should be shown by actual test to be harmless.

While the exact mode of reaching the end in view is somewhat different from the one suggested above, yet the fundamental object, that each batch of color used in foods shall be specifically tested, and that such colors shall be harmless, is attained with reasonable certainty; and although there are colors other than these seven which are undoubtedly equally as little objectionable, and while it is true that the present policy contemplates the permitted use of but seven specific colors, yet that policy, as before outlined, is sufficiently broad and elastic to enable the addition of a color to the permitted list, when it is shown that such color really fills a need, not properly satisfied by one of the colors already permitted or some combination of these, and is in and of itself harmless. There can be no objection to the expansion of the list to such an extent as to include every harmless coal-tar color in existence; but the burden of proving such real need and harmlessness is very properly placed upon those who are seeking such expansion.

As far back as 1892 the following statement was made on page IV of the Leffmann translation of Weyl's book on coal-tar colors, in connection with the various European legislative enactments: "It is certain that none of these plans is even approximately satisfactory and the problem will be even more difficult of solution in the United States; indeed, it seems to me to be unsolvable." In view of this opinion the results of the food inspection decisions as herein shown may properly be regarded as, at least, a step in the right direction toward the solution of this problem.

This opinion is further supported by C. A. Neufeld who, in reviewing Food Inspection Decision No. 76, says: "The idea of permitting only specific selected coloring matters for use in the production of articles of food, and of excluding all other colors from such uses, must, in the interest of control of articles of food, be regarded as an extraordinarily happy one; a similar regulation is to be urgently recommended for our own country." (*Zts. Nahr. Genussm., 1908, v. 15, p. 434.*)

IDENTITY OF COAL-TAR COLORS USED IN FOOD PRODUCTS IN THE UNITED STATES IN 1907.

COLLECTION OF SAMPLES.

The question "Which coal-tar colors shall be permitted for use in coloring such food products as are to be consumed within the United States?" can be answered, "All colors now in use, or to be used for that purpose, provided they are harmless and necessary as defined on page 14."

This involves the further questions:

1. Which coal-tar colors of the 695 different chemical individuals now on the world's markets are actually used in the United States for that purpose?
2. If restricted to such coal-tar colors as are now in use in the United States for this purpose, would this be likely to hamper or interfere with the invention of other coal-tar colors suitable for the coloring of food?

It would be physically impossible to go to every user of coal-tar colors in food products in the United States and obtain specimens of the coal-tar colors so employed; this would be impracticable not only because of the large number of such users, and their wide geographical distribution, but also because they often do not know what they are using, and further because of a reluctance, undoubtedly to be encountered among many, to disclose the nature of the products employed. This is rendered more than likely by the attitude of some of the makers of coal-tar colors, or their accredited agents, as will be shown later.

However, the sources of coal-tar colors are limited in number. By reference to pages IX and X of "A systematic survey of the organic coloring matters," by Arthur G. Green, published in London and New York by Macmillan & Co. (Ltd.), in 1904 (hereinafter referred to as "Green Tables"), it will be seen that there are approximately 37 different concerns the world over engaged in the manufacture of coal-tar colors. Therefore a canvass of these sources for such coal-tar colors as in their judgment, or in their business practice, they regard as proper for use in food products, is the best way of arriving at a fair demarcation of the field of coal-tar colors here in question.

Communication was therefore had with 13 actual manufacturers of coal-tar colors, in an endeavor to obtain from them such coal-tar colors as in their judgment or business practice are suitable for use, or are used in food products.

A request was also made for information as to the chemical composition of the coal-tar color specimens submitted; in order to avoid confusion, it was further asked that reference be made to the Green Tables, in which each chemical individual or coal-tar color has its own number, and if any of the contributed specimens was not so listed that the chemical composition be stated in a manner analogous to that used in the Green Tables. This procedure was necessary in order to reduce the terminology to a common and nonequivocal basis. Out of the 13 makers, or their accredited sole importers or selling agents in the United States, who were consulted, 9 have supplied the specimens requested; the remaining 4 promised to contribute, but have not done so. In the following table is shown the amount and character of the information obtained:

Tabulation of distribution of replies and character of information received.

Country.	Geographical distribution.				Number of samples contributed.	Number of samples referred to Green Tables.	Number of samples not referred to Green Tables.	No composition or ambiguous terminology.
	Coal-tar color makers.	Makers asked to send samples.	Makers not sending samples.	Makers sending samples.				
Germany	16	6	....	6	181	106	1	74
England	8	1	....	1	12	12	....	....
France	5	1	1	....	....	....	....	....
Switzerland	5	3	3	....	....	....	....	....
United States	1	2	....	2	61	35	5	21
Holland	1	....	....	....	....	....	....	....
Belgium	1	....	....	....	....	....	....	....
Total	37	13	4	9	254	153	6	95

In order to make provision for the 24 makers listed in the Green Tables and not included in the 13 makers addressed requests for samples were sent to two domestic houses which import coal-tar colors from scources other than the above, for use in food products; their products must fairly represent any of the colors not covered by the 13 makers addressed. Of these two importers, one responded with 13 samples, and of each he gave the number in the Green Tables corresponding to each specimen; the other importer has not redeemed his promise to contribute specimens.

A third importer volunteered the Green Table numbers of four out of five coal-tar colors used in his business, but could not even approximately say what the remaining color was chemically. He did not contribute any specimens, nor was that necessary at the time this information was volunteered.

A fourth importer contributed specimens of five coal-tar colors needed in his business, but was able to give Green Table numbers for only three of them; he could not give even approximately the chemical composition of the remaining two.

Out of the 17 responsible concerns consulted 5, or 29 per cent, have not found it to their interest to contribute either specimens or information.

CLASSIFICATION OF SAMPLES SUBMITTED.

GREEN TABLE NUMBERS.

Out of the 284 specimens contributed, or reported on, 172 (60.6 per cent) were identified as to their chemical composition, by reference to the Green Tables; 6 (2.1 per cent) were otherwise unequivocally identified chemically, and for 106 (37.3 per cent) the makers, or their responsible agents, declined to state the chemical composition, i. e., 62.8 per cent were unequivocally identified, and the remaining 37.3 per cent were not so identified.

The specimens submitted are therefore divisible into the following three classes:

Class I. Those for which numbers were given in the Green Tables, numbering 172.

Class II. Those whose composition was given in chemical language, numbering 6.

Class III. Those whose composition was not given in any language capable of correct and certain translation into chemical terms, numbering 106.

Consider Class I. The Green Tables, page VI, divide the coal-tar colors into 21 groups, comprising 695 different chemical individual coal-tar colors. The 172 members of Class I number in all 74 individuals, or 10.6 per cent of the Green Tables, and fall into 11 of the 21 groups of those Tables.

The following table classifies the samples according to the Green Table groups:

Green Table groups and number of collected samples falling within them.

Color groups of the Green Tables.	Number of members in group.	Collected samples falling into the several groups.		Color groups of the Green Tables.	Number of members in group.	Collected samples falling into the several groups.	
		Number.	Per cent.			Number.	Per cent.
Nitro	6	1	16	Anthracene	37		
Monoazo	126	30	24	Indophenol	8		
Disazo	204	11	5	Azin	38	2	5
Trisazo	46			Oxazin	32		
Tetrakisazo	11			Thiazin	9	2	22
Nitroso	5	1	20	Thiazol	6		
Stilbene	18			Quinolin	4	1	25
Oxyketone	8			Sulphid	21		
Diphenylmethane	2	1	50	Indigo	7	1	15
Triphenylmethane	66	15	23				
Xanthene	35	9	26		695	74	10.6
Acridin	6						

SOURCE.

The distribution of the 74 different chemical individuals of Class I among the 12 different sources from which they were obtained is as follows:

Distribution of the 74 different samples of Class I among the 12 sources supplying same.

Number.	Per cent.	Number of sources from which each came.	Number.	Per cent.	Number of sources from which each came.
35	47.3	1	1	1.35	7
20	27	2	1	1.35	8
4	5.4	3	0	....	9
4	5.4	4	1	1.35	10
6	8.1	5	0	....	11
3	2.7	6	0	....	12

It follows from this table that there is very little unanimity among the different concerns furnishing coal-tar colors for use in food products as to which of their products are desirable, necessary, or suitable for such use.

Inspection of this table shows that only three colors out of 74, or 4 per cent, were wanted by more than half of all the sources; that only 6, or 8.1 per cent, were wanted by half of the sources; and that not one of the colors was wanted by all the sources. This last statement is true of manufacturers as well as importers, each group taken by itself.

PATENTS.

This lack of unanimity is not due to the patent situation, because not more than one of these 74 products is patented, and it is more than likely that the United States patent on this product has long since expired.

Moreover, only 6 of the 12 sources offered colors at one time patented by themselves or others. The total number of such ex-patented products is 45, and of these only 22 were offered by those who had patented them; the remaining 23 were offered by sources other than the ex-patentees, and were not offered by such ex-patentees.

Patented colors.

Total number of patented products offered.	Ex-patented products offered by patentee.	Total number of patented products offered.	Ex-patented products offered by patentee.
11	2	1	1
16	8	4	2
6	5		
7	4	45	22

It would, therefore, seem to be rather clear that others think more favorably of such ex-patented products as food colors than do the original patentees. In view of the fact that the latter would generally be in a better position, and would have greater opportunity than any one else to judge of the suitability of the patented products for use in food products, it may well be inferred that such products are not altogether free from disadvantages as food colors.

The second of the two questions propounded, namely, If restricted to such coal-tar colors as are now in use in the United States for this purpose, would this be likely to hamper or interfere with the invention of further coal-tar colors suitable for the coloring of food products? can be answered "No" because none of the colors submitted was discovered later than 1891; out of the 214 coal-tar colors since then discovered not one was among those submitted for use in foods, and out of the 481 discovered in 1891 and prior thereto, only 74 were so submitted, or 2 out of every 13 of such colors. In the following table these data are given year by year:

Coal-tar colors discovered from 1740 to 1891.

Year of discovery.	Number of submitted colors discovered.	Total number of coal-tar colors discovered.	Year of discovery.	Number of submitted colors discovered.	Total number of coal-tar colors discovered.	Year of discovery.	Number of submitted colors discovered.	Total number of coal-tar colors discovered.	Year of discovery.	Number of submitted colors discovered.	Total number of coal-tar colors discovered.
1740	1	1	1871	1	6	1881	4	11	1887	1	29
1856	1	3	1874	1	4	1882	5	20	1888	1	36
1859	1	1	1875	6	12	1883	9	25	1890	1	46
1861	2	3	1876	5	9	1884	3	16	1891	3	33
1862	1	5	1877	2	10	1885	2	21			
1863	1	3	1878	12	26	1886	3	32	Total.	74	378
1867	1	3	1879	7	23						

SHADES OF COLOR.

The sufficiency of the 74 colors used for food-coloring purposes in the United States, for any and all tinctorial ranges, no matter how refined, appears from the following table:

Green Table numbers of the 74 submitted colors showing shades and number of sources supplying each.

Shades.	Number of sources out of a possible 12 offering each color.										Total number of dyes offered for each shade.
	1	2	3	4	5	6	7	8	9	10	
Red	104	65			106	103	107				12
	105	108									
	169	146									
	240	462									
	584										

Green Table numbers of the 74 submitted colors showing shades and number of sources supplying each—Continued.

Shades.	Number of sources out of a possible 12 offering each color.										Total number of dyes offered for each shade.
	1	2	3	4	5	6	7	8	9	10	
Blue red		502 518 520 523		448	504 517						7
Yellowish red	54 516	512									3
Scarlet	53 64	55									3
Bluish scarlet	56										1
Yellow	89 269 329			510	8	94				4	7
Reddish yellow	9	84									2
Orange yellow		14				13					2
Greenish yellow	667		425								2
Orange	18 97	17 85 95						86			6
Blue	439 476 480 655	650	692								6
Green blue	440										1
Violet	468	452			451						3
Blue violet	464										1
Grayish violet	287										1
Green	433 434	398		435							4
Blue green		427									1
Yellow-green			428								1
Brown	101 137 139										3
Reddish brown		201		197							2
Blue black	188										1
Blue to bluish red to violet, according to brand	601										1
Oil-soluble colors	10 49 60	11									4
Total	35	20	4	4	5	3	1	1		1	74

The six colors comprising Class II (those not listed in the Green Tables but whose composition was avowed or disclosed) are one black, one yellow, and one orange among the water-soluble colors, and three yellows among the water-insoluble colors. Therefore two-thirds of the United States market as thus disclosed calls for a total of 80 different chemical individuals, of which 73 are water-soluble and 7 are water-insoluble and are used as oil or fat colors. The remaining third of the makers or dealers either do not possess the information or are unwilling to give it. No attempt has yet been made to enter systematically into this unknown region, but careful examination warrants the belief that it can add nothing of material value to the data already obtained which show a total of 23 shades for the 73 water-soluble coal-tar colors, summarized as follows:

Number of coal-tar colors.

5 red shades	26	3 green shades	6
4 yellow shades	14	2 brown shades	5
1 orange shade	7	1 blue-black shade	1
2 blue shades	7	1 black shade	1
3 violet shades	5	1 blue to violet shade	1

The water-insoluble colors numbering 7 are not included, but will be treated separately (p. 159).

This view of the state of the United States market at or about the middle of the year 1907 is without question a true reflection of that market as far as it goes and the actual extent of the coal-tar color market beyond those covered by this canvass of it is not likely to be very great. In support of this view is the interchangeable treatment of formerly patented products, the great lapse of time since a new food color was discovered, and the fact that out of the 74 colors submitted and contained in the Green Tables only 23 are now less than 25 years old, and none is less than 16 years old.

All the Green Table numbers and the number of sources out of a possible 12 offering them are given in the following table:

Number of sources, out of a possible 12, offering colors designated in 1907.

Green Table No.	Sources.	Green Table No.	Sources.	Green Table No.	Sources.	Green Table No.	Sources.
[1] *4*	10	86	8	240	1	476	1
8	5	89	1	269	1	480	1
9	1	94	6	287	1	502	2
10	1	95	2	329	1	504	5
11	2	97	1	398	2	510	4
13	6	101	1	425	3	512	3
14	2	103	6	427	2	516	1
17	2	104	1	428	3	*517*	5
18	1	105	1	433	1	518	2
49	1	106	5	434	1	520	2
53	1	*107*	7	*435*	4	523	2
54	1	108	2	439	1	584	1
55	2	137	1	440	1	601	1
56	1	139	1	448	4	650	2
60	1	146	2	451	5	655	1
64	1	169	1	452	2	667	1
65	2	188	1	462	2	*692*	3
84	2	197	4	464	1		
85	2	201	2	468	1		

[1] Italicized figures indicate colors permitted by F. I. D. 76.

Nine manufacturers sent 261 specimens, an average of 29 each, distributed as follows: 70; 38; 20; 20; 15; 43; 25; 18, and 12. Two importers sent 5 specimens each, and one 13, a total of 23 specimens, and an average of 8. These figures reflect a diversity of opinion as to what is needful for food coloring, since each one of these 12 makers or importers believed that for all practical food-coloring purposes his selection was complete and sufficient.

Classifying the 284 specimens as red, yellow, brown, orange, blue, green, violet, and black, the following table is obtained showing the different requirements of each of the 12 makers or importers to produce the necessary shades of the eight colors mentioned:

Total specimens submitted, grouped by makers and colors, showing number of shades required by each.

Maker's number.	Red.	Yellow.	Brown.	Orange.	Blue.	Green.	Violet.	Black.	Number of shades wanted by each.	Total specimens.
1	25	18	14	4	2	5	2	......	7	70
2	10	6	2	1	3	1	2	......	7	25
3	7	5	......	2	1	1	1	1	7	18
4	11	10	10	5	2	2	3	......	7	43
5	6	2	......	3	4	2	2	1	7	20
6	4	3	2	1	3	1	1	......	7	15
7	6	2	1	2	1	......	......	......	5	12
8	5	4	......	1	1	1	1	......	6	13
9	6	4	1	3	......	4	2	......	6	20
10	13	10	2	3	5	4	1	......	7	38
11	3	1	......	1	......	......	......	......	3	5
12	3	1	......	......	......	1	......	......	3	5
Total	99	66	32	26	22	22	15	2	......	284
Per cent	34.86	23.24	11.27	9.15	7.75	7.75	5.28	0.70	......	100
Maximum	25	18	14	5	5	5	3	1	......	......
Minimum	3	1	......	......	......	......	......	......	......	......
Average	8.25	5.50	2.75	2.17	1.83	1.83	1.25	0.17	......	......
Permitted	3	1	......	1	1	1	......	......	......	......

From this table it appears that not one of the 12 sources desired all of the 8 shades into which the 284 specimens are classifiable to make up a complete set of food colors; 7 out of the 12 sources wanted 7 of the 8 shades; 2 sources wanted 6 out of the 8 shades; 1 source wanted 5 of the 8 shades, and 2 sources were content with 3 out of the 8 shades.

It will be noticed that the permitted list given in Food Inspection Decision No. 76 provides for 7 dyes covering 5 out of the 8 shades of the above classification. The 3 missing shades are brown, violet, and black; the shades provided are red, yellow, orange, and blue.

It will also be noticed that on the whole 6 out of the 8 shades were not wanted by one or more of the 12 sources. The italicized shades are the ones not provided for by the permitted list of Food Inspection Decision No. 76.

Brown was not wanted by 5, nor orange by 1, blue by 3, green by 2, *violet* by 3, nor *black* by 10.

The combinations not wanted were as follows: Five sources omitted *black* only; 2, *brown* only; 1 blue and *black;* 1 *brown* and *black;* 1 green, *violet*, and *black;* and 1 *brown*, blue, green, *violet*, and *black*.

Not one of these 12 sources wanted only the three colors not found on the permitted list, and 2 sources did not want any of the three missing shades nor two of the permitted colors.

In view of this large difference of opinion among the 12 sources as to the shades needed to make a complete set of food colors, the 5 shades selected for the permitted list of Food Inspection Decision No. 76 seem reasonably close to any consensus of opinion derivable from the tabulation of the collected facts.

II. PURPOSES OF FOOD COLORING.

The use of any color which conceals inferiority, or which gives an article an appearance better than it properly possesses is, of course, illegitimate, and such cases are not here considered. Among such uses may be mentioned that of color in pastry to impart a yellow color thereto, implying the presence of eggs, when they are either wholly absent or are not present in sufficient quantities to produce a shade of color which would indicate a superior quality. Such coloring is frequently resorted to in macaroni, spaghetti, noodles, and the like, and it has also been stated in the literature that such coloring has the additional function of concealing dirt actually present in the flour.

The addition of red coloring matter to meat products to give them an appearance of freshness which they do not of themselves possess; the addition of red coloring matter to strawberry, raspberry, and similar jams, jellies, and preserves, to give them a color indicative of exceptional quality, even though they may contain none of the fruit whose presence is intimated by the label on the product; the injection of red coloring matter into ordinary oranges to give them the appearance of blood oranges; the sprinkling of lemons and oranges with green coloring matter to give them the appearance of a particular origin or of a particular state of ripeness when such origin or state of ripeness is without foundation in fact; the injection of red coloring matter into watermelons to give them the appearance of ripeness, which ripeness they do not possess, are practices met more or less frequently.

Among the purposes for which food colors are said to be used and the foods so colored, the following are mentioned in the literature:

In European countries.

1. Macaroni is colored with Dinitrocresol (2) (*Arch. Pharm., 3d ser., v. 22, p. 621*) and Martius Yellow (3) (*Weyl, Handbuch*).
2. Cordials and liqueurs with Dinitrocresol (2) (*Arch. Pharm., 3d ser., v. 22, p. 621*).

3. Oranges: Biebrich Scarlet (163) (*Weyl, Handbuch*).
4. Pastry: Dinitrocresol (2) (*Weyl, Handbuch*).
5. Butter: Dinitrocresol (2) (*Weyl, Handbuch*).
6. To whiten flour: Anilin blue (457) (*Zts. Nahr. Genussm., 1906, v. 12, p. 298*).
7. Noodles are colored to cover up cigar butts, burnt matches, mineral oil, etc. (*Zts. Nahr. Genussm., Vol. II, p. 1018*).

In the United States.

8. Jellies, fruit sirups, soda sirups, jams, ketchup, cheap cordials, lemon extract, milk, butter, cheese, ice cream, confectionery, pastries, flavoring extracts, mustard, cayenne pepper, sausage, noodles, wines, and liqueurs (*Winton, Connecticut Agricultural Experiment Station Report, 1901, pp. 179–182*).
9. Cattle feed is colored yellow (*Gudeman, J. Amer. Chem. Soc., 1908, v. 30, p. 1623*).
10. "Egg color" (399); "Macaroni color" (94); "Tomato catsup color" (105); "Raspberry color" (103); "Mustard color" and "Pie filling color" (4); "Orange color" (87), and "Strawberry red color" (55) are corresponding United States commercial food color names and their corresponding Green Table numbers (*Meyer, J. Amer. Chem. Soc., 1907, v. 29, p. 895*).

Dr. E. Ludwig, of Vienna, stated, upon the authority of Dr. Schacherl, at the International Congress of Medicine held in Budapest, in August, 1909, as follows:

The rather widely distributed practice of coloring baker's goods yellow, such as cakes and the like, further the yellow coloring of pastry, macaroni, noodles, and so forth, has as its function the representation of a very large egg content in them; this coloring has been made very convenient because there are in commerce colors intended specifically for this purpose and designated "egg substitute" and which have nothing whatever in common with egg yolk.

Marmalades such as apricot, raspberry, and currant marmalades are frequently found in a colored condition in commerce; in this case the purpose of the coloring is frequently to cover up adulteration; the adulteration may consist in an admixture of a cheap fruit pulp, particularly apple pulp, or in an addition of glucose sirup. Since these admixtures do not possess the color of the marmalades they are simply helped along by the aid of color.

Old fruit sirups are toned up with color and then sold as fresh sirup.

Red colored fermentation vinegar and red colored vinegar essence as well as vinegar made from such essence are in commerce; such coloring has for its purpose to represent the product as "genuine red wine vinegar," which in some countries is highly desired.

So-called "beer color," said to be an extract of roasted malt (malt caramel), is in fact nothing but ordinary sugar caramel and is frequently from time to time publicly advertised; breweries themselves do not use this preparation, but it has been frequently shown that in small taverns by means of this color local beer was converted into Bavarian beer.

The wholesale coloring of coffee beans serves the purpose of representing a better quality than it actually is.

The coloring of cocoa and chocolate by the use of mineral additions and also of coal-tar colors was often proven; in this case the coloring serves exclusively to cover up poor quality. In the case of good products such coloring is not practiced.

Colored sausages, and in fact such with a colored meat body as well as such with a colored casing, are frequently colored; coal-tar colors and cochineal serve this purpose, the latter, however, only for the meat. This coloring is to preserve in old goods the appearance of fresh goods.

The green canned goods of commerce are almost all colored with copper compounds.

Tomato pulp frequently comes into commerce colored with a coal-tar color; the purpose of such coloring is to impart to the goods the appearance of having been prepared with extraordinary care. In all these cases it is not at all a question of a harmless change of the natural condition of the food product, but of improper manipulations which are adapted to deceive the purchaser as to the real value of the goods; even, indeed, to mask the danger to health.

III. FOOD-COLOR REQUIREMENTS.

ADAPTABILITY FOR SPECIAL PURPOSES.

Not all coal-tar colors are adapted for use in food products. Colors are the more desirable for this purpose the higher their tinctorial power, and the greater the resistance they offer to the action of the materials with which they are to be used, and under the conditions existing. Obviously only such colors as of themselves have their tinctorial properties fully developed can be used, and all such colors as require a mordant to develop or bring out the color are not fit for nor capable of use in food products.

Further, if the colored material is subjected to varying temperatures in the process of manufacturing foods, it should be able to withstand the effects of such temperatures, as, for example, in the manufacture of candies. The colors should also withstand the action of reducing agents, such as are generated in the course of fermentation and decomposition of the food product, or where a preservative such as sulphur dioxid is added to the food product to minimize the effect of decomposition of the food upon the color. Such colors are put on the European market, and perhaps, but not necessarily, on the United States market with preservatives added to them. Most of the coal-tar colors are susceptible to the action of sulphur dioxid, particularly when the latter has been used in the decolorizing of glucose, and Uranin (510) is one of the colors found to have the greatest resistance to the sulphur dioxid which may remain combined in candy.

For example, the book entitled "Henley's Twentieth Century Book of Receipts, Formulas and Processes," published in 1907, on page 359, says of sausage color:

> It is absolutely necessary in using aniline colors to add a disinfectant to the dyestuff solution, the object of which is, in case the sausage should commence to decompose, to prevent decomposition of azo-dyestuff by the disengaged hydrogen. Instead of boracic acid, formaline may be used as a disinfectant.

J. Fraenkel (*Arbeit. Kaiserl. Gesundh. 1902, v. 18, pp. 518–521;* abst. *Zts. Nahr. Genussm., 1902, v. 5, p. 986*) reports as follows on the composition of colors used in coloring sausages, meats, and preserves:

1. Blood color: Moisture, 15 per cent; common salt, 6.6 per cent; borax, 21 per cent; and Ponceau 2 R (G. T. 55).

2. Blood red for meat juices: Liquid of a specific gravity of 1.0163 not affected by acids or alkalis and containing 27 per cent total solids; of these total solids 31 per cent were salt, 12 per cent borax, and the remainder Ponceau 2 R (G. T. 55).
3. Casing red: This powder contained Orange II (G. T. 86).
4. Sausage red: A liquid containing Eosin.
5. Lobster color: A liquid of specific gravity 1.0064 containing 1.64 per cent of solids, of which 10.9 per cent were salt and the remainder Ponceau R T (G. T. 44).

G. Possetto (*Zts. Nahr. Unters. Hygiene Waarenk. 1891, v. 5, p. 105*) cited the following 15 colors as being used for the coloring of pastry:

	G. T. No.		G. T. No.
Martius Yellow	3	Tropæolin 00	88
Victoria Yellow	2	Tropæolin 000 No. 1	85
Naphthol Yellow S	4	Tropæolin 000 No. 2	86
Aurantia Yellow	6	Chrysoidin	17 18 41
Acid Yellow G	8		
Acid Yellow R	9		
Citronin	91	Azoflavin	92
Tropæolin 0	84		

Algerian Saffron (a mixture of Nos. 4 and 86 and crocein).
Prussian Saffron (composition not given).

"Blood-red" on the American market is starch colored with red coal-tar colors (*Ibid., 1896, v. 10, p. 114*).

"Butter yellow" is a clear saponifiable oil of reddish-yellow color, containing 3 per cent of anilin-azo-dimethylanilin (No. 16 of the Green Tables).

PROPORTION OF COAL-TAR COLOR USED.

The amount or proportion of coal-tar color used has been variously stated. On page IV of the Leffmann translation of Weyl's book entitled "The Sanitary Relations of the Coal-Tar Colors," it is stated that 1 ounce of Auramin (G. T. 425) will color 2,000 pounds of confectionery, which means 1 part of color in 32,000 parts of colored product.

Frentzel (*Zts. Nahr. Genussm., 1901, v. 4, pp. 968–974*), on authority not given, says that for sirups 1 part of color is used to from 4,000 to 5,000 parts of sirup; in colored sugars 1 part of coloring matter to from 1,333 to 4,000 parts of sugar; and in flour 1 part of coloring matter to from 666 to 1,000 parts of flour.

In pastry 1 to 100,000 parts (*Zts. Nahr. Unters. Hygiene, Waarenk. 1893, v. 7, p. 34*).

In chapter VII (p. 47), sections 15, 16, 17, and 18, are brought together statements made before the commission on regulations for the Federal food and drugs act, as to the amount of color contained in colored food products. Briefly these are as follows: Confectionery, 1 part of color in 3,500 parts of product; beverages 1 part of color in 128,000 parts, 256,000 parts, 1,024,000 parts; butter, 420 grains of color to 1,000 pounds of butter; or 1 part of color to 16,666 parts of butter.

One ounce of color to 30 pounds of "colored food;" the colored food was not further defined; which means 1 part of color in 480 parts of colored product.

It has further been represented that 1 part of color is sufficient to whiten 250,000 parts of yellow sugar.

From time to time others have presented information as to the amount of color used in food products. All of these data available have been tabulated, showing the number of parts of colored product containing 1 part of coal-tar color, arranged in the order of the amounts present:

Food [1]	100
Do [2]	480
Flour	666
Do	1,000
Sugar	1,333
Confectionery	3,500
Sirups	4,000
Sugar	4,000
Sirups	5,000
Confectionery	12,800
Butter	16,666
Confectionery	20,000
Confectionery	24,576
Do	30,000
Do	32,000
Beverages	80,000
Pastry	100,000
Beverages	120,000
Do	128,000
Confectionery	192,000
Whitening sugar	250,000
Beverages	256,000
Do	1,024,000

Grouped according to the kind of material colored, the ranges given are as follows:

Beverages	80,000; 120,000; 128,000; 256,000;1,024,000
Butter	16,666
Confectionery	3,500; 12,800; 20,000; 24,576; 30,000; 32,000; 192,000
Flour	666; 1,000
Food	[1] 100; [2] 480
Pastry	100,000
Sugar	1,333; 4,000
Sirups	4,000; 5,000
Whitening sugar	250,000

These statements have emanated from persons presumably acquainted with the facts of their own practice, and if that presumption is correct it appears that there are wide variations in practice not only among individual users, but for individual colors. No attempt has been made to prove or disprove these statements by actual determination of the amount of color contained in commercial colored food products.

The 80 chemical individuals on this market for food-coloring purposes, it can be fairly assumed, have been tested and tried out as to their utility, and in this respect further tests were regarded as superfluous and therefore have not been undertaken.

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[1] Kind not definitely stated.

[2] Said to be for preserved tomatoes.

SUITABILITY OF SHADES OF PERMITTED COLORS AND MIXTURES OF SAME.

The shades produced by the seven permitted colors are, respectively, yellow, orange, blue, green, red, bluish scarlet, and brilliant cherry red. As statements are found in the literature against the use of all of the chemical individuals producing a brown or a violet shade, it will be necessary to produce these shades by a proper combination of two or more of the permitted colors. So far no criticism with regard to the shades produced by the seven colors themselves and by their appropriate mixture has been made that has been substantiated.

Objection has been made to the violet producible from blue and red, on the ground that when applied to a food product, such as candy, the component parts do not evenly fix themselves upon the material. This objection, however, has not been pressed and probably is not well taken, because of the fact, frequently reported, that few, if any, coal-tar colors are used without admixture of one or more other colors to shade or to tone the original color. This criticism, therefore, of the use of mixed colors can be regarded as not a serious objection.

One criticism urged with considerable persistency against the seven permitted colors was that none of them would withstand the action of the organic acids ordinarily found in beverages such as lemonade, and it was suggested that no color was proper for use for such purposes which would not withstand, unaltered, for a period of 12 hours the action of a 10 per cent solution of citric acid. In urging this objection substitutes were suggested for the permitted colors. The substitutes so urged were Tartrazin (94), Azorubin (103), Orange II (86), Ponceau 4 GB (13), and one other color designated as Scarlet SR, of whose chemical composition no information whatever was forthcoming. (The numbers in parentheses refer to the Green Tables.) Of the five colors suggested it can be said that concerning all but No. 103 adverse statements are found in the literature, and No. 86 is specifically regarded by every observer but one as being thoroughly poisonous. The suggested substitute list is, therefore, objectionable on the ground of injuriousness to health.

To test the validity of the assertion that none of the permitted colors could withstand the action of citric acid, solutions of the suggested colors, as well as of the permitted colors, each one in a thousand, were submitted to the action of citric acid, added in such quantity that it amounted to 10 per cent of the total bulk of solution. This experiment showed that Tartrazin is reddened by citric acid, whereas Naphthol Yellow S loses in tinctorial power to a slight extent. The shade produced by Ponceau 4GB can be closely imitated by a mixture of Naphthol Yellow S, Orange I, and Amaranth, all permitted colors. There is no choice whatever in the shade produced by the desired Azorubin and the permitted Amaranth, nor is there any difference in behavior toward citric acid. The difference in the shade between the desired Orange II and the permitted Orange I is so small that it requires a side-by-side comparison to distinguish between them. Moreover, the desired Orange II produces a precipitate when brought in contact with the citric acid, whereas the permitted Orange I does not so precipitate. The permitted Erythrosin is, of course, completely precipitated by the citric acid. The permitted Light Green and Indigo disulphoacid are weakened in tinctorial power by the addition of the citric acid. Of these colors the only ones used to any extent in beverages, so far as either the suggested or permitted list is concerned, are red, yellow, green, and orange.

As has been shown the permitted reds equal the desired reds and the permitted orange is better than the desired orange. The tinctorial power of the permitted yellow is not so great as the tinctorial power of the desired yellow, but this difference is so slight that the objection urged against the list of permitted colors, namely that they were so poor in quality that they had destroyed a profitable and lucrative business in the coloring of beverages, is untenable in view of the fact that, assuming a price of $1 per pound for Tartrazin, and 40 cents for Naphthol Yellow S, and using them in the proportions necessary to produce a lemonade color in a 10 per cent citric acid solution, it would take 5,000 quarts of finished lemonade to cause an increase of 1 cent in the cost of the production of the colored food product; that is, it increases the price per quart by one five-thousandth of a cent.

It has also been urged that the permitted green is not good enough for cordials and liqueurs, and that it is impossible to bring about the proper green by the use of the permitted yellow and blue. This criticism, however, has not been persisted in; the fact is that mixtures of the permitted yellow and blue can be made so as to obtain any desired shade of green, having a yellow or blue cast, and great clarity and brilliancy. How these mixed colors would look after a long period of time has not been ascertained.

It has also been said that the permitted red, Amaranth, is not a color suitable for the coloring of strawberry jams and it has been urged that the same chemical individual under another commercial name is better than the permitted red. This criticism has not been pressed, probably for the reason that it can not be substantiated.

Again it was claimed that the deposits to be noticed in bottled lemonades were due to Naphthol Yellow S, but solutions of Naphthol Yellow S in citric acid have remained without deposit for upward of 15 months; it is possible that such precipitation, if observed, may be due to an admixture of the nonpermitted Orange II with Naphthol

Yellow S, and this difficulty can be obviated by the use of the permitted Orange I, which does not precipitate in the citric acid, as shown.

Naphthol Yellow S has been objected to on account of the bitter taste it is said to impart to the beverages to which it is added, and steps have finally been taken by those interested to have another yellow placed on the permitted list.

It has also been objected that the permitted blue is not suitable for the coloring of sugar, first, because it is soluble, and second, because of the unsatisfactory shade. It may be sufficient, in answer to this criticism, to state that there was no insoluble blue offered on this market, and there was no blue other than the one permitted offered against which adverse statements did not exist in the literature, and in view of this state of affairs the criticism may be said to be not well taken.

As against all these specific criticisms it has been repeatedly stated by those in a position to know that they have found no difficulty whatever, by suitable mixtures of permitted colors, in reproducing any desired shade of any desired quality, not even excepting browns and violets.

Considering all of these criticisms, therefore, the conclusion seems reasonable that there is no serious or permanent objection to be made against the seven colors selected, either as to qualities for food-coloring purposes or range of producible shades.

IV. CONFORMITY OF FOOD-COLOR MARKET, 1907, TO RECOMMENDATIONS OF THE NATIONAL CONFECTIONERS' ASSOCIATION, 1899.[1]

Having thus shown that the food-color market of the United States contains not less than 80 coal-tar colors which are distinct chemical individuals, of which 74 are entered in the Green Tables and 6 are not, the next question to be considered is whether all of these substances are harmless and fit for use in food products.

As a guide in determining this point the "Official circular from the executive committee of the National Confectioners' Association of the United States," pertaining to colors in confectionery, dated February 1, 1899, may well be considered.

The function of this circular is said to be "to throw light upon the vexed question of what colors may be safely used in confectionery," evidently because "there may at times be a doubt in the mind of the honest confectioner as to which colors, flavors, or ingredients he may safely use and which he may reject."

The circular also states that "but infinitesimal amounts of color (coal-tar colors) need be or can be used to give the desired effects," and in view of this statement as to quantity it must be self-evident that a color harmful when used in the small quantities said to be used in confectionery is certainly harmful when used in the large quantities used in coloring other food products. This circular under the heading, "Colors that are injurious and therefore to be rejected—Harmful organic colors," enumerates 21 coal-tar colors. Of these 21 colors, 13, or 61.9 per cent, were among those submitted, and whose composition was stated by reference to Green Table numbers; the Green Table numbers of these colors, together with the number of sources from which they were obtained, follow:

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[1] See also p. 45.

Green Table numbers.	Number of sources.	Green Table numbers.	Number of sources.
11	2	169	1
17	2	197	4
18	1	201	2
84	2	398	2
86	8	584	1
95	2	650	2
106	5		

Out of these 13 colors 3 each came from one source; 7 each came from two sources; 1 came from four sources; 1 came from five sources, and 1 came from eight sources, out of a possible 12; that is, one was wanted by more than half the sources.

It is further to be noted that of the trade names given to the submitted products of Class I the following appeared among the harmful list of this circular and also were found in identical form and spelling on the labels of the submitted products:

1. Bismarck Brown.
2. Chrysoidin R.
3. Chrysoidin Y.
4. Mandarin G extra.
5. Naphthol Green B.
6. Napthol Yellow.
7. New Coccine.
8. Orange A.
9. Orange A extra.
10. Orange G.
11. Orange II.
12. Scarlet.
13. Vesuvin B.
14. Crocein Scarlet 5 B.

The following parallel will serve to show the great resemblance between the names given to the harmful colors of the circular and those found on the samples submitted:

Circular's harmful list.	*Labels of submitted samples.*
Methylene Blue B B	Methylene Blue B.
Methylene Blue B B crystals	Methylene Blue D.
Methylene Blue B B G	Methylene Blue O.
Methylene Blue D B B	
New Coccine	New Coccine O Z. New Coccine Z.
Naphthol Green B	Naphthol Green.

Circular's harmful list.	*Labels of submitted samples.*
Orange II	Orange II Z.
Victoria Yellow	Victoria Yellow Conc. Z.
	Victoria Yellow Conc. T Z.
Acid Yellow	Acid Yellow G.
Bismarck Brown G	Bismarck Brown B.
Bismarck Brown T	Bismarck Brown B 216.
	Bismarck Brown Dark.
	Bismarck Brown R X.
	Bismarck Brown T D.
	Bismarck Brown Y Bril.
	Bismarck Brown Y Dark.
	Bismarck Brown 2 R X.
Chrysoin	Chrysoin Brown G Z.
	Chrysoin G E Z.
	Chrysoin R E Z.
	Chrysoin R Z.
Cochineal Red A	Cochineal Red.
Crocein Scarlet 3 B	Crocein Scarlet 10 B.
Crocein Scarlet 7 B	
Crocein Scarlet 8 B	
Fast Brown G	Fast Brown N.
	Fast Brown O.
Fast Yellow	Fast Yellow G.
	Fast Yellow O 3 3.
	Fast Yellow O 3 4.
	Fast Yellow Y.
Imperial Scarlet, in powder, extra	Imperial Scarlet 3 B.
Safranin	Safranin S P.
Safranin A G extra.	
Safranin AGT extra.	
Safranin Conc.	
Safranin extra G.	
Safranin FF extra No. 0.	
Safranin G extra GGS.	
Safranin G 000.	
Safranin T.	

This comparison disclosed a considerable lack of conformity between the United States food-color trade in 1907 and the circular of February, 1899, upon whose preparation for seven months prior to its date "a great deal of thought and labor have been given to a thorough investigation of the whole subject of 'colors in confectionery,' in which the committee has been largely aided by the researches of the association's chemist and by the results of his analytical tests" and whose "classifications have been carefully made, and are based upon the authority of the eminent chemists, Prof. Koenig and Prof. Weyl, upon the resolutions of the Swiss chemists and upon the French ordinances regarding the coloring of food products," and which list was expected to be "of value to color dealers and chemists," and also was published to assist the confectioner in obeying "the letter and the spirit" of the pure-candy laws.

Under the heading "Colors that have been shown to be harmless as used in the confectioner's art, harmless organic colors," this circular enumerates 36 colors, for 4 of which there are no Green Table numbers. Of the 32 colors having Green Table numbers, 20, or 62.5 per cent, were among those colors submitted. The Green Table numbers of these colors, together with the number of sources from which they were obtained, follow:

Green Table numbers.	Number of sources.	Green Table numbers.	Number of sources.
4	10	240	1
8	5	269	1
9	1	287	1
13	6	427	2
55	5	448	4
65	2	451	5
85	2	462	2
103	6	512	3
105	1	517	5
107	7	520	2

Of these 20 colors 5 each came from 1 source; 5 each came from 2 sources; 1 came from 3 sources; 1 came from 4 sources; 4 each came from 5 sources; 2 each came from 6 sources; 1 came from 7 sources, and 1 came from 10 sources; that is, only 2 were wanted by more than half the makers or importers, and only 4 by half the sources.

It will be noted that this circular provides for a total of only 57 different coal-tar colors; the number of avowed colors submitted reached 80, or 23 in excess of this number, and further, that out of the 57 colors referred to in this circular only 33 appeared among those colors submitted whose composition was acknowledged, so that for 47, or 58.8 per cent of the avowed submitted colors, this circular is no specific guide. From the data obtainable from this circular, the following tabulation can be made:

Harmfulness of submitted colors based on list in circular.

Data.	Total on market.	Disclosed colors on United States market.	Circular's corresponding list.
		Per cent.	*Per cent.*
Harmful	13	16.3	61.9
Harmless	20	25.4	62.5

These figures disclose a considerable and self-evident disregard of the request, then eight years old, as made by the National Confectioners'

Association of the United States, that coal-tar colors designated by it as "colors that are injurious and therefore to be rejected, harmful organic colors" be not used in confectionery, and by implication that they should not be used in other food products.

In view of this disregard with respect to 13 coal-tar colors out of 33 on the United States market in the summer of 1907, dealt with in this confectioners' list, the conclusion seemed justified that the action regarding other coal-tar colors on the United States market in the summer of 1907 was equally heedless or indifferent, a conclusion which is borne out by the material brought together under section IX.

The necessity of adhering to some unequivocal terminology, as has been done in these pages by referring to the serial numbers in the Green Tables, appears from the following:

Trade names are not definite with respect to the composition of the article sold under a given name, although tinctorially the colors may be substantially equivalent. In the Green Tables there are not less than 29 instances where the same trade name is applied to two or more different chemical individuals. In some cases these chemical individuals are fairly closely related, in others they are only remotely related. When the differences are greater than the presence or absence of a sulpho group or the use of methyl for ethyl or the reverse, the instances are given below:

1. Cotton Yellow.........	128	Primulin-azo-m-phenylene-diamin-disulphonic acid.
	191	Diphenylurea-disazo-bi-salicylic acid.
2. Methyl Eosin..........	513	Methylated tetrabromo-fluorescein.
	375	Dinitro dibromo fluorescein.
3. New Yellow..........	88	p-sulphanilic acid azo-diphenylamin.
	91	Nitration product of diphenylamin yellow.
4. Orange III...........	23	Meta-nitranilin azo-R salt.
	87	p-sulphanilic acid azo-dimethylanilin.
5. Orange N..............	43	Toluidin azo-Schaffer acid.
	88	p-sulphanilic acid azo-diphenylamin.
6. Orange R..............	97	o-toluidin-monosulphonic acid azo-betanaphthol.
	15	Anilin-azo-R salt.
	99	Xylidin sulphoacid-azo-betanaphthol.
7. Toluylene Red.......	261	Dichloro-benzidin disazo R salt.
	580	Dimethyl diamido toluphenazin.

The following quotation also bears on this point:

> In attempts to group the aniline colors a kind of uncertainty appears even among color chemists. The same trade name does not always correspond to the same preparation. Many preparations are not chemical individuals, but mixtures of related colors. Many preparations are "standardized" for the trade; for example, with dextrin. On account of the patent laws, factory secrets surround the production of many coloring matters, and frequently statements are met with which are directly intended for the purpose of misleading competition. (*Hueppe, Die Methoden der Bakterienforschung, 5th ed., 1891, p. 105.*)

V. SOME LEGAL ENACTMENTS RELATIVE TO THE USE OF COAL-TAR DYES.

LIST OF THIRTEEN FOREIGN LEGAL ENACTMENTS.

The 13 foreign legal enactments compared are as follows:

1. The Austrian regulation of March 1, 1886, which forbids No. 483 of the Green Tables and all anilin colors.
2. The law of Austria of May 1, 1886, which forbids No. 1 of the Green Tables and all anilin colors.
3. The Austrian regulation of September 19, 1895, in which certain colors only are permitted, and all others are forbidden. There are 16 titles of permitted colors in this law, but these are in some cases so elastic and so indefinite as to include 47 entries in the Green Tables.
4. The Austrian law of January 22, 1896, in which there are 17 titles, but these are sufficiently elastic to allow of 22 entries in the Green Tables being included in them.
5. The German law of July 5, 1887, which specifically prohibits only Nos. 1 and 483 of the Green Tables. The interpretation which seems to be generally placed upon this law is that all other Green Table members are permitted in Germany for use in foods.
6. The Italian law of February 7, 1892, forbidding all colors except 9 different titles, which, however, were elastic enough to include 32 entries in the Green Tables.
7. The law of Italy of February 7, 1902, which prohibits 37 entries in the Green Tables, and permits 11 specifically.
8. The Italian decree of June 29, 1893, in which there were 7 titles of permitted colors sufficiently elastic to include 34 different individuals.
9. The Italian decree of March 24, 1895, forbidding four titles covering only four entries in the Green Tables.
10. The French police ordinance of May 21, 1885, in which 489 entries in the Green Tables were prohibited.
11. The French police ordinance of December 31, 1890, in which 469 entries of the Green Tables were prohibited, and which also permitted under 9 titles 23 entries in the Green Tables.
12. The Belgian law of 1891, which specifically forbids only four entries in the Green Tables.
13. The law of the Canton of Tessin, dated May 18, 1897, which forbids only 4 specific entries in the Green Tables.

SUMMARY OF COLORS PERMITTED BY THESE LEGAL ENACTMENTS.

An examination of the 13 legal enactments made in Europe with respect to the use of coal-tar colors in food products discloses considerable difference of opinion as to the harmfulness or the harmlessness of even the same chemical individuals. To prepare an approximate and comprehensive summary of the effect of such legislation, the following plan has been followed:

The provisions of a selected number of laws and regulations, 13 in number and dating from 1883 to 1902, were read with respect to permission or prohibition of the 695 chemical individuals listed in the Green Tables. The effect of each law upon each separate entry in the Green Table numbers was noted, either as permitted, forbidden, or noncommittal when the law was silent upon such entry; the laws were read with the understanding that what was not forbidden was permitted, and what was neither forbidden nor permitted was noncommittal; if this were an incorrect or improper procedure the number of permitted colors would be reduced but not augmented. For the purposes of this first approximation, no attempt was made to segregate those specifically permitted from those permitted by blanket expressions or phrases.

For the purposes of a side-by-side comparison, the term "index number" was coined; this "index number" gives in the first place the number of legal enactments that permit the color; in the second place the number of enactments that forbid it; and in the third place the number of enactments that are silent or noncommittal. Thus: 283 as an index number would mean 2 enactments permit, 8 forbid, and 3 are noncommittal; so that an index number with the highest hundreds would have the greatest number of permissions, and with the highest tens would have the highest number of prohibitions. With this understanding of these terms, the following table of so-called index or "P. F. N." numbers is offered:

Thirteen legal enactments classified by Green Table numbers and the "P. F. N. figure," or "index number."

Total number of Green Table entries.	Index number.	Green Table numbers.
1	184	483.
1	274	534.
3	2,10,1	1-3.
217	364	394-411, 415-426, 429-434, *435*[1], 436-445, 484-492, 528-561, 564-583, 585-598, 600, 602-649, 651-691, 693-695.
1	373	103.
366	382	6, 7, 10, 11, 12, 14, 16, 19-22, 35-40, 42, 43, 45-54, 58-64, 66-83, 89, 90, 93, 94, 96-101, 104-106, 109-113, 115-145, 149, 151-156, 158, 159, 161, 162, 164-168, 172, 197, 199-243, 245-393, 412-414, 493-511, 524-527, 650.
29	391	446, 447, 449, 450, 453, 455, 456, 458-461, 463-476, 478, 479, 481, 482.
3	454	428, 563, 599.
1	463	*692*.
22	472	*5*, 8, 9, 23-34, 84, 86-88, 91, 520, 523.
7	481	95, 452, 454, 477, 513, 519, 522.
2	544	562, 601.
2	562	515, 516.
3	571	480, 514, 521.
3	643	44, 114, 427.
11	652	13, 15, *85*, 92, 108, 146-148, 150, 160, *517*.
6	661	17, 18, 41, 169, 512, 518.
2	670	451, 462.
6	742	*4*, 55. *56*, 57, 102, 244.
6	751	65, *107*, 163, 170, 198, 448.
1	850	457.
2	931	157, 171.

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[1] Italicized figures indicate colors permitted by F. I. D. 76.

From the table it appears that there is no one Green Table number that is permitted by each and all of these 13 legal enactments, nor is there any one color that is prohibited by each and all of these 13 legal enactments; and as late as 1902 there was considerable con fusion as to what should or should not be permitted or forbidden. That the European enactments were not in reality consistent or effective appears from the following:

1. In respect to the use of coal-tar colors, the views as to their harmfulness or harmlessness are very divergent, and this uncertainty is expressed in the various legislative enactments. (v. Raumer, *Zts. Nahr. Unters. Hygiene & Waarenk., 1895, v. 9, p. 207.*)
2. After eating groats, which no doubt were free from ordinary poisons but had been colored with Martius Yellow, a whole family became sick. Since this coloring matter is not mentioned among those which, under the law of July 5, 1887, are forbidden for the purpose of coloring articles of food, a complaint could not be lodged. Nevertheless, the use of Martius Yellow for the coloring of articles of food would seem to be dangerous, since this coloring matter exerts poisonous effects. It is a weakness in the law that coal-tar colors, of which new members are continuously appearing on the market, and whose physiological action is unknown, should be at all permitted for the coloring of articles of food. (Dietrich, Th., *Jahresberichte der landwirthschaftlichen Versuchsstation, Marburg, 1900–1901, p. 13;* abst. *Zts. Nahr. Genussm., 1902, v. 5, p. 364.*)

On account of their large number and the great diversity of opinion as to harmfulness of some coal-tar colors and the harmlessness of others therein reflected, no attempt was made to collect all the legal enactments and regulations made with respect to coal-tar dyes. The foregoing are typical of the remainder.

COLORS SAID TO BE PERMITTED UNDER THE GERMAN LAW OF 1887.

That some of the laws did not employ specific terms but used those possessed of a great degree of elasticity appears from the following taken from Weyl's "Sanitary relations of the coal-tar colors," page 38, concerning the Chamber of Commerce and Trade of Sonneberg which declared on December 4, 1887, that the German law of July 5, 1887, allowed the unrestricted use of—

All blue and violet anilin (that is coal-tar) colors, all ponceaus, all orange colors, Methyl Green, Brilliant Green, Malachite Green, Chrysoidin, Naphthol Yellow, Martius Yellow, Eosin, Phloxin, Safranin, Erythrosin, Fuchsin, Phenylene Brown, and Anilin Black.

This amounts to not less than 233 permitted colors, as will now be shown.

Using the Green Tables as a guide it will be found that under the above ruling there are to-day no less than 107 blue coal-tar colors which could be used for food coloring. They are arranged as follows, showing the comments on same in the literature.

BLUE COLORS.

Unfavorable: 478, 479, 488, 490, 572, 602, 639. (Total, 7.)

Favorable: 477, 599, 600, 692. (Total, 4.)

Contradictory: 287, 457, 480, 563, 601, 650, 689. (Total, 7.)

Not reported on: 36, 83, 119, 142, 175, 189, 209, 246, 247, 254, 257, 263, 266, 288, 290, 291, 292, 293, 294, 295, 301, 302, 310, 311, 314, 315, 316, 317, 318, 319, 323, 327, 345, 347, 348, 351, 352, 356, 359, 364, 430, 432, 439, 440, 442, 444, 456, 473, 476, 481, 482, 487, 492, 509, 542, 551, 556, 558, 559, 560, 562, 569, 595, 598, 608, 611, 612, 615, 618, 619, 621, 625, 627, 628, 634, 637, 638, 640, 641, 642, 643, 652, 655, 656, 657, 664, 682, 693, 694. (Total, 89.)

Similarly there would be 50 violets which are classified as follows:

VIOLET COLORS.

Unfavorable: 620, 649. (Total, 2.)

Favorable: 467, 593. (Total, 2.)

Contradictory: 450, 451. (Total, 2.)

Not reported on: 30, 34, 36, 118, 176, 179, 207, 244, 246, 248, 252, 256, 273, 320, 336, 338, 340, 342, 343, 444, 452, 454, 455, 463, 464, 465, 466, 468, 469, 470, 471, 472, 474, 486, 506, 507, 525, 552, 579, 581, 585, 592, 613, 625. (Total, 44.)

Similarly there would be 18 ponceaus which are classified as follows:

PONCEAUS.

Unfavorable: None.

Favorable: 169, 448. (Total, 2.)

Contradictory: 13, 15, 55, 160, 163. (Total, 5.)

Not reported on: 44, 56[1], 57[1], 108, 113, 114, 146, 147, 148, 150, 165. (Total, 11.)

Similarly there would be 35 Oranges which are classified as follows:

ORANGES.

Unfavorable: 2, 97. (Total, 2.)

Favorable: 85. (Total, 1.)

Contradictory: 14, 18, 43, 86, 87, 88, 95. (Total, 7.)

Not reported on: 10, 23, 47, 54, 99, 100, 136, 162, 196, 217, 218, 222, 225, 235, 236, 265, 275, 392, 406, 408, 409, 529, 531, 545, 547. (Total, 25.)

The remaining 23 colors named are classified as follows:

MISCELLANEOUS.

Unfavorable: Martius Yellow (3).[2] (Total, 1.)

Favorable: Naphthol Yellow S. (4); Eosin (512, 517, 521); Phloxin (521); Erythrosin (517). (Total, 6.)

Contradictory: Brilliant Green (428); Malachite Green (427); Chrysoidin (17, 18, 41); Safranin (584); Fuchsin (448); Phenylene Brown (197). (Total, 8.)

Not reported on: Methyl Green (460, 461); Eosin (514, 515); Phloxin (518); Safranin (583, 585); Erythrosin (516); Anilin Black (577). (Total, 9.)

It will be noticed that names such as Eosin, Erythrosin, and Phloxin appear in more than one classification; thus some of each are favorably reported on and the others are not reported on at all.

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[1] Nos. 56 and 57 are included among the permitted colors of the Austrian law.

[2] The numbers in parentheses following the names are the Green Table numbers.

The 233 colors said by the Sonneberg Chamber of Commerce and Trade to be entitled to unrestricted use in food coloring under the German law of 1887 referred to may also be classified as follows:

Summary of classification of colors permitted under the German law of 1887 according to comments in the literature.

Class.	Total.	Comments in the literature.			
		Unfavorable.	Favorable.	Contradictory.	Not reported on.
Blues	107	7	4	7	89
Violets	50	2	2	2	44
Ponceaus	18	0	2	5	11
Oranges	35	2	1	7	25
Miscellaneous	23	1	5	8	9
Total	233	12	14	29	178

Out of these 233 colors only 55, or about one-fourth, have been reported on in the literature and the remaining three-fourths have not been examined at all. To the 55 examined and reported on there may be added 2, namely, Nos. 56 and 57, since they are included among the colors once permitted by law in Austria, thus making a total of 57 examined out of 232. Adding these two to the 14 favorably reported on makes a total of 16. It finally appears that 41 out of 57 colors examined would in the light of present knowledge be improper to be used in food and 12 at least should not be used for such purposes at all.

The classification into unfavorable, favorable, contradictory, and not reported on is based upon the tabulation on page 63; if that is substantially correct the above conclusions are also true.

The foregoing side-by-side comparison of 13 legal enactments, while it makes no claim to being absolutely and wholly accurate in all the classifications or conclusions drawn, is no doubt a fair reflection of the condition of mind of those framing the enactments, and consequently of information upon which those enactments were based; and the conclusion would therefore seem to be justified that the fact that a color has been permitted or has been forbidden by any one or the majority of these legal enactments ought not to constitute a clean bill of health, nor an indictment, as the case may be.

This side-by-side comparison must not be pushed to extremes; indeed the extent to which it can be employed is naturally very limited, and the purpose for which it was made was to reflect in a manner easily and comprehensively grasped the confusion and inconsistencies which very persistently force themselves upon the mind of a person reading those enactments and having in mind the chemical individuals at which they are aimed.

The definite lesson to be learned from this side-by-side comparison is that these enactments in many cases employed terms so vague and indefinite as to permit the use of some bad colors as well as all good ones, that is they were not sufficiently definite to exclude all that were harmful.

DEFINITENESS AND DETAIL NECESSARY TO EFFECT QUALITY CONTROL.

This apparent state of confusion in legal enactments that preceded the summer of 1907 was a very strong factor in the formation of the conclusion that in order to be effective any law or regulation dealing with coal-tar colors for use in foods must prohibit every coal-tar color except certain definite specific ones.

The Austrian laws of September 19, 1895, and of January 22, 1896, provided for quality control by public and other laboratories of the coal-tar colors put upon the market for use in foods; the results of such control, as reported in the Zeitschrift für Nahrungsmittel-Untersuchung, Hygiene und Waarenkunde, 1896, v. 10, p. 335, are as follows:

> Coloring matters of commerce are mostly mixtures of various coloring matters, a right which manufacturers will not part with; and further, while it is indeed possible to test the coloring matter in substance, it is nevertheless impossible to test it in the very small amounts which are used in the coloring of foodstuffs and to determine with certainty the identity of the color as to whether it is or not one of the permitted colors.
>
> Of 21 samples of coloring matter examined, 14 were objectionable, partly because of false labeling, or because they were mixtures, partly because they contained poisonous metals, or a forbidden coloring matter. Thus, a so-called "Evergreen" was Naphthol Green B, a poisonous nitroso color; Malachite Green contained zinc; an Acid Magenta and a Rosalin contained traces of copper; Ponceau, Eosin, Brown, and Roccellin contained traces of tin; Orange I and Waterblue contained traces of tin and zinc. The last-named coloring matters were therefore not prepared in proper state of purity.
>
> The authorities in Vienna examined four and rejected two colors. (*Ibid., 1898, p. 107.*)

The Swiss authorities exercised control over colors, after they reached the market, with the result that the authorities in Basle examined ten colors and rejected one. (*Ibid., 1897, p. 292.*)

These facts, together with the knowledge derived by even the most superficial ocular examination of the 294 specimens received in the summer of 1907, played a very great part in the formation of the conclusion that control of quality, in order to be even reasonably effective, must be thoroughgoing, and that colors must be excluded from the market until they prove themselves to be clean, rather than permitted promiscuously and then driven out of the market by the authorities if unsuitable.

The effective quality control of food colors requires careful and searching examination of a kind which can not usually be obtained by the general purchasing public. The quality of the food colors offered in the summer of 1907 varied greatly, and the substances contaminating them were of such indefinite and probably variable composition (of whose physiological action nothing definite was known and whose quick and certain detection in the colored food product would be very complicated, if not impossible) that quality control of greater efficiency than that exercised by those selling food colors in the summer of 1907 seemed necessary on the part of the authorities having charge of the enforcement of the food and drugs act.

The points of original entry of food colors into the United States food-color market are relatively few, whereas the points of distribution of food colors are very many, the former being less than 20 and probably fewer than 10, while the latter may number up into the hundreds; therefore, not only is the labor and the expense of quality control of food colors reduced to its probable minimum by keeping food colors off the market until they have shown their right to be so used, but also the certainty and the efficiency of quality control is increased to its probable maximum.

The quality control thus suggested is similar to that exercised by the States of New York, Michigan, and Ohio over salt before it enters the market for human consumption. The method of color control here suggested differs only in degree, not in kind, from the quality control exercised over salt by the States named. Experience has shown such quality control of food colors to be not only practicable but capable of realization without any hardship and but little, if any, inconvenience to those concerned.

STATE LAWS PROHIBITING THE USE OF COLORS IN CERTAIN FOODS, 1909.

The laws of the individual States of the United States have also restricted the use of coal-tar coloring matters in foods. These restrictions are directed principally against the use of color to conceal inferiority, which restriction is found in almost all the States.

The sale of poisonous coloring matters for foods is prohibited in the State of New York, and in New York and North Carolina the addition of injurious colors to foods is prohibited.

Minnesota and North Carolina prohibit coal-tar dyes in all foods.

Foods and beverages are considered adulterated in North Dakota and Wyoming if they contain aniline dyes or other coal-tar dyes.

Artificial coloring is prohibited in sausages by Colorado and Wisconsin.

Artificial coloring, including, of course, coal-tar colors, must not be added to vinegar in the States of Arkansas, California, Connecticut, Iowa, Minnesota, Missouri, New Jersey, New York, Pennsylvania, Tennessee, Wisconsin, and Wyoming.

Distilled vinegar must not contain artificial color in Ohio and Oklahoma, and must be free from harmful artificial coloring matter in Utah.

In South Dakota oleomargarine must not be colored.

Artificial coloring is prohibited in milk by California, Oklahoma, Pennsylvania, Utah, and Wisconsin and in cream by California Connecticut, Pennsylvania, Utah, and Wisconsin.

Coal-tar dyes are inhibited in cakes, crackers, candy, ice cream, and like products by Virginia. Ice cream is considered adulterated in Michigan if it contains harmful colors.

Forty-six States prohibit the use of poisonous colors in candy. They are as follows: Alabama, Arkansas, California, Colorado, Connecticut, Delaware, District of Columbia, Florida, Georgia, Idaho, Illinois, Indiana, Iowa, Kansas, Kentucky, Louisiana, Maine, Maryland, Michigan, Minnesota, Missouri, Montana, Nebraska, Nevada, New Hampshire, New Jersey, New York, North Carolina, North Dakota, Ohio, Oklahoma, Oregon, Pennsylvania, Philippine Islands, Porto Rico, Rhode Island, South Carolina, South Dakota, Tennessee, Texas, Utah, Vermont, Virginia, Washington, Wisconsin, and Wyoming.

VI. RECOMMENDATIONS BY ASSOCIATIONS AND INDIVIDUALS AS TO USE OF COAL-TAR DYES AS FOOD COLORS.

CAZENEUVE AND LÉPINE.

Cazeneuve and Lépine (*Bull. de l'acad. de médicine, April 27, 1886, p. 643*) says:

> We have arrived at the following conclusions:
>
> 1. The nitro derivatives are especially poisonous (dinitronaphthol being comparable with picric acid), but the sulphonated product is harmless.
>
> 2. Safranin and Methylene Blue are harmful, producing gastric intestinal disturbances, being violent poisons.
>
> 3. The following coloring matters are tolerated by man, whether well or affected with Bright's disease; similarly, too, animals (dogs, guinea pigs) without any noticeable disturbances and at rather high doses:

	Probable Green Table Nos.		Probable Green Table Nos.
1. Fast Yellow	9	5. Ponceau R	55
2. Roccellin	102	6. Orange I	85
3. Bordeaux B	65	7. Fuchsin S	462
4. Purple	106 or 107		

> Among the nontoxic sulphonated colors we have been able to make out the following list or classification, based upon their power of producing disturbances, proceeding from the least inert to the most inert:

	Probable Green Table Nos.		Probable Green Table Nos.
1. Orange I	85	5. Yellow NS	4
2. Bordeaux B	65	6. Fast Yellow	9
3. Ponceau R	55	7. Purple	106 or 107
4. Roccellin	102		

The results of our experiments have led us to the following conclusions:

1. The relative nonpoisonous nature of the azo colors used for coloring wines explains why this artificial coloring has not caused any real epidemic.

2. This artificial coloring of wines by coal-tar colors is dangerous. It opens the door to the employment of coloring matters of very variable and noxious properties. Thus Martius Yellow, which is poisonous, has been used for 10 years past to color pastry (3 grams per 100 kilograms) and it may be used to-morrow, perhaps, to color wines mixed with a red or a blue.

3. A rigid law against the artificial coloring of wines ought to be promulgated, particularly if this coloration covers detestable practices most prejudicial to the public health. The addition of salicylic acid, glycerin, and tartaric acid, or the acidifying by sulphuric acid, is cloaked by the use of the coloring matter.

Some sulphonated azo-coloring matters are sufficiently inert to enable their being employed as artificial color in foods, bonbons, and liquors. These colors are manufactured according to simple processes which give theoretical yields and no metallic salt, such as mercury, tin, or arsenic participates; sulphate of soda is the only impurity.

In view of the great extent of the use of these coloring matters, it is better to regulate their consumption by tolerating certain of these products rather than to interpose an illusory barrier to their use. Where you can not arrest a stream you can at least regulate its course.

It would be better definitely to classify these substances with respect to their noxious properties, tolerate some and prohibit the others, rather than to be exposed to the consequences of permitting manufacturers to introduce into food, without any scientific control whatever, any products whatever.

These coloring matters should be sold in commerce under the names of harmless colors as determined by analysis. By chemical analysis it would be recognized as to whether we were dealing with one color or with a mixture of two or three colors.

The colors most used are made up of red, yellow, and blue, which apparently imitate the appearance of the wines of the Midi. Thus we have recognized such coloring matters in Roccellin, Naphthol Yellow, and Methylene Blue.

Sulphonated Fuchsin is very much used, combined with a yellow and a blue. This mixture turns green with ammonia, like the coloring matter of wine. In fact, Acid Fuchsin is decolorized by ammonia. The yellow and blue remain intact, and give a green which suggests true wine color.

SOCIETY OF SWISS ANALYTICAL CHEMISTS.

In 1891 the Society of Swiss Analytical Chemists recommended that certain coloring matters which are to be regarded as harmful to health should not be permitted to be used in the preparation of articles of food intended for sale in which artificial coloring is at all permitted.

The coal-tar colors thus prohibited are identified in the following by their Green Table numbers, only one trade name being given: Picric Acid (1); Dinitrocresol (2); Martius Yellow (3); Aurantia (6); Orange II (86); Metanil Yellow (95); Safranin (584); Methylene Blue (650). (*Zts. Nahr. Unters. Hygiene, 1891, v. 5, p. 293.*)

TSCHIRCH.

In 1893 Tschirch recommended as follows:

1. The coal-tar colors, and in a narrow sense the anilin colors, are no longer harmful on account of their arsenic content, since at the present time the great majority of them are prepared free from arsenic.

2. Some colors have shown themselves to be harmful to the system.

3. Coal-tar colors in general should therefore be permitted for the coloring of foods, but those that have been found to be harmful should be expressly and specifically forbidden.

4. The amount of coloring matter which has been determined quantitatively in bonbons and liqueurs is so small that even the ones regarded as poisonous would not be able to develop their harmful effects. (*Zts. Nahr. Unters. Hygiene Waarenk., 1893, v. 7, p. 338.*)

KAYSER.

In 1895 Kayser expressed himself as follows:

As to the poisonous nature of organic coloring, and in respect to their composition, H. Erdmann (*Pharm. Centralh., 1892, v. 33, p. 357*) concludes that in general acid dyestuffs can pass as nonpoisonous; whereas in the case of basic coloring matters it is recommended to make a physiological examination before using them for the coloring of things in daily use, especially articles of food. Whether that portion of that view which deals with acid dyestuffs will retain unexceptionable and positive validity appears doubtful.

At the present time, speaking generally, interested manufacturers take the point of view that all the coloring matters which are not forbidden in the food law are to be regarded as permitted. Whether this point of view is free from legal objection can not be discussed here; that, however, it can not be accepted from a hygienic point of view under any circumstances whatever does not require any special proof for those conversant with the facts. The hygienic requirements under all circumstances can be summed up in the following rule:

Every coloring matter is to be regarded in every way as suspicious, so far as its harmlessness is not proven by experience or by correct physiological experiments.

No one can say in advance that among the colors which are to-day manufactured and used, which are as yet not called into question, there are none which possess distinctly poisonous properties. Correct examination of artificial coloring matters in this direction is, as is well known, even to the present almost wholly lacking. (*Forschungsberichte über Lebensmittel*, etc., *1895, Vol. II, p. 181.*)

WEYL.

In 1896 Weyl expressed himself as follows:

Since the number of the organic coloring matters already known is a very large one, and since their number is increasing daily, and it seems to be unlikely that each individual of these coloring matters will be examined as to its poisonous nature, there are only two ways left in which to solve the question as to the use of coloring matters in the manufacture of food and articles of daily use.

One of them, and at the same time the simplest, would be to prohibit the use of all coloring matters for the coloring of foods, etc. This rigorous point of view will hardly ever be taken by legislators, because it would be tantamount to the removal of many marks of differentiation which have become desirable and necessary.

The second way seems to be the much more practicable, and which Theodor Weyl proposed some time ago.

It consists in permitting the use of only a definite number of coloring matters, recognized as harmless, for the coloring of articles of food, etc. Which coloring matters are to be so permitted is to be determined by the authorities having jurisdiction. The same authorities are also to determine the maximum amount of each coloring matter which can be used for any purpose. New coloring matters can be used only for the above-mentioned purposes when they have been recognized as nonpoisonous after official test. All permitted coloring matters must be also detectable, even in small amounts. (*Handbuch der Hygiene, 1896, Vol. III, p. 385.*)

NATIONAL CONFECTIONERS' ASSOCIATION.

In 1899 the National Confectioners' Association of the United States issued an official circular, which has been previously discussed,[1] designating certain colors as harmful, and certain others as harmless; the members of each class are given in the following list, in which only one trade name is given, the Green Table number appearing in parentheses at the end of that name.

HARMFUL ORGANIC COLORS.

Red colors: Ponceau 3RB (163); Crocein Scarlet 3B (160); Cochineal Red A (106); Crocein Scarlet 7B (169); Crocein Scarlet O extra (164); Safranin (584).

Yellow colors: Picric Acid (1); Martius Yellow (3); Acme Yellow (84); Victoria Yellow (2); Orange II (86); Metanil Yellow (95); Sudan I (11); Orange IV (88).

Green colors: Naphthol Green B (398).

Blue colors: Methylene Blue BBG (650).

Brown colors: Bismarck Brown (197); Vesuvin B (201); Fast Brown G (138); Chrysoidin (17, 18).

HARMLESS ORGANIC COLORS.

Red colors: Artificial Alizarin and Purpurin (534); Eosin (512); Erythrosin (517); Rose Bengale (520); Phloxin (521); Ponceau 2R (55); Bordeaux B (65); Ponceau 2G (15); Fuchsin S (462); Archil Substitute (28); Orange I (85); Congo Red (240); Azorubin S (103); Fast Red D (107); Fast Red (105); Ponceau 4GB (13); Fuchsin (448).

Yellow and Orange colors: Naphthol Yellow S (4); Brilliant Yellow (5); Fast Yellow (8); Fast Yellow R (9); Azarin S (70); Orange (43).

Green colors: Malachite Green (427); Dinitrosoresorcin (394).

Blue colors: Indigo (689); Gentian Blue (457); Coupiers Blue (600).

Violet colors: Paris Violet (451); Wool Black (166); Azoblue (287); Mauvein (593).

Brown colors: Chrysamin R (269).

SCHACHERL.

Schacherl in 1903 made the following statement:

> If coal-tar colors are to be permitted for the coloring of food, then, in my opinion, it is not right to limit the use of such to a few coloring matters, but groups of coloring matters must be permitted which are without suspicion from a sanitary standpoint, and which are characterized by definite reactions. Other groups, on the other hand, which contain harmful or merely suspicious colors, must be absolutely excluded. * * * The selection would be easily made if sufficient data were at hand with respect to the physiological action. Unfortunately this is lacking, a circumstance which need not be surprising in view of the very large number of synthetic coloring matters, since the Schultz-Julius tables enumerate 681 such colors. Unfortunately the experiments of Th. Weyl, which were planned on a large scale, have not been completed, and apart from isolated investigations we are limited in passing judgment upon the most of these colors to the proof that to date nothing with respect to harmful action has become known. * * * Consequently it is still possible that one or the other coloring matter which may to-day be regarded as above suspicion, or a newly discovered coloring matter may possess poisonous properties; the legislators should under all circumstances have the power to exclude for use in foods all suspicious combinations, and all such coloring matters as are not easily distinguishable from them.

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[1] See also p. 30.

The final recommendations of Schacherl amount to permitting—

1. All the Azo colors, Nos. 7 to 393 of the Green Tables, except No. 86.
2. All the Triphenylmethane colors, Nos. 427 to 492 of the Green Tables, except the hydroxyl derivatives, which would be Green Tables Nos. 483, 484, 485, 486, and 491.
3. All Pyronins, Nos. 493 to 527 of the Green Tables.
4. All Oxyketones, Nos. 537 to 570 of the Green Tables.
5. All Indulins, Nos. 599, 601, and 603 of the Green Tables.
6. Naphthol Yellow S, G. T. No. 4.
7. Methylene Blue, G. T. No. 650.

The use of all other coal-tar colors would best be forbidden, partly from hygienic and partly from practical considerations (rendering control more easy), until the absolute harmlessness of the group in question is determined by physiological test. * * * It should be required of all permitted coloring matters that they shall not contain substances which are harmful to health, or even suspicious, either in chemical union or as contaminations. (*Fifth International Congress of Applied Chemistry, Berlin, 1903, Vol. IV, pp. 1041–1048.*)

The exclusions recommended are all nitro-colors, except Naphthol Yellow S; all acridin colors and all chinolin colors; Auramin, Indophenol and all nitro-colors, except Naphthol Yellow S, Schacherl regards as not necessary; further, he has no knowledge of the physiological action of any of the azoxy or the thiobenzenyl colors, and aside from Methylene Blue, he has no knowledge of the physiological action of the oxazins and thiazins.

CLASSIFICATION OF RECOMMENDATIONS IN THE LITERATURE.

The following table shows the groups of coal-tar colors of the Green Table classification and the Green Table numbers of the members of each of the groups, together with the favorable or unfavorable recommendations found in the literature in regard to each and a statement as to those regarding which no recommendations are made:

Tabulation of recommendations found in the literature.

Group number.	Green Table number.	Name of color.	Number of individuals reported on—			Total number.	
			Unfavorably. (1)	Favorably. (2)	Conflictingly. (3)	Reported. (4)	Not reported. (5)
1.	1–6.	Nitro	4	2	0	6	0
2.	7–182	Monoazo	3	10	19	32	94
3.	133–336.	Disazo	3	3	7	13	191
4.	337–382.	Trisazo	......	......	......	......	46
5.	383–393.	Tetrakisazo	......	......	......	......	11
6.	394–398.	Nitroso	0	1	1	2	3
7.	399–416.	Stilbene	0	1	0	1	17
8.	417–424.	Oxyketone	......	......	......	......	8
9.	425–426.	Diphenylmethane	1	0	0	1	1
10.	427–492.	Triphenylmethane	5	5	8	18	48
11.	493–527.	Xanthenes	2	5	1	8	27
12.	528–533.	Acridin	2	0	0	2	4
13.	534–570.	Anthracene	0	0	1	1	36
14.	571–578.	Indophenols	2	1	0	3	5
15.	579–616.	Azins	2	3	2	7	31
16.	617–648.	Oxazins	2	0	0	2	30
17.	649–657.	Thiazins	3	0	1	4	5
18.	658–663.	Thiobenzenyl	1	0	0	1	5
19.	664–667.	Quinolin	1	0	0	1	3
20.	668–688.	Sulphids	2	0	0	2	19
21.	689–695.	Indigos	0	1	1	2	5
		Total	33	32	41	106	580

CONCLUSIONS.

Applying the Schacherl rule, "Other groups which contain harmful or merely suspicious colors must be absolutely excluded," to this table and assuming that all entries in columns 1 and 3 shall be regarded as rendering such colors as "harmful or merely suspicious," it will be found that only one group, namely the Stilbene group, would be permitted under that rule; further, that this rule would admit 17 colors, not one of which has been reported on in the literature as to its physiological action. This state of affairs tends to emphasize the difficulties in the way of any generalization which will be safe so far as public health is concerned and fair to those who use food colors for admittedly legitimate purposes and to make the following recommendation appears to be the only satisfactory way of solving the food-color problem:

> Although it would be possible to draw quite reliable conclusions as to the advisability of employing certain colors for food products on the basis of their chemical constituency, the mode of their manufacture and of the ingredients used in same, nevertheless, I think that by far the safest way would be on the one side to force the dealers of colors intended for food products to sell only such colors with which exhaustive and careful physiological tests have been made by experienced and especially impartial and thoroughly reliable people, thereby establishing their harmlessness beyond a doubt. On the other hand, the manufacturers and canners of food products of any description should be forced to purchase and use only those colors which they are sure have been submitted to such careful tests as have been described and by these tests found to be harmless. (*Lieber, The use of coal-tar colors in food products, 1904, p. 150.*)

This view is confirmed by Santori[1] (*Moleschott's Untersuchungen, 1895, Vol. XV, p. 57*), who says:

> From all these experiments it follows that it is impossible, as some have desired to do, to conclude simply from the chemical composition and constitution whether any given coal-tar dye is poisonous or nonpoisonous. Thus Indulin belongs to the same group as Printing Blue and Methyl Violet to the same group as does Acid Violet; therefore each individual coal-tar dye must be separately examined, and it is only by this laborious method that the use of all really poisonous coal-tar dyes will be prevented.

VII. RECOMMENDATIONS MADE BY UNITED STATES COLOR INDUSTRIES AND TRADES TO THE DEPARTMENT OF AGRICULTURE.

Prior to the issuance of any regulations, the commission on rules and regulations for the food and drugs act, June 30, 1906, held meetings in New York City during September of that year. The stenographic reports of those meetings, and the briefs filed, in so far as they relate to colored food, colored food products, or material for coloring foods or food products, have been condensed verbatim in the following pages with the sole exception of omissions, as indicated; for obvious reasons the names of those making the suggestions hereinafter quoted are not given. This review of opinions expressed and recommendations given by the industries and trades most interested in the manufacture, use, and sale of food colors and colored food products is believed to be fair and full with respect to each and every quotation. The numbers in parentheses following each quotation refer to the pages in the stenographic minutes of the hearing from which those quotations are made.

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[1] Santori examined 15 different blue and violet dyes on dogs by the mouth and hypodermically. Of these 15, 8 are poisonous by the mouth and 7 are nonpoisonous by the mouth. He found Indulin to be poisonous and Printing Blue to be nonpoisonous; Acid Violet to be nonpoisonous and Methyl Violet to be poisonous.

ANTAGONISTIC TO ALL ADDED ARTIFICIAL COLOR.

1. Our position in the coloring question is that we are opposed to all artificial coloring matter in food products. * * * (p. 109).

2. Secondly, speaking first of our own business, and I believe that my view would hold as regards all food products, it is my opinion that all added artificial coloring matter in food products should be prohibited. My experience in our line of business demonstrates this to me beyond any question of doubt (p. 439).

CONCERNING RESTRICTIONS AND REQUIREMENTS.

WHAT CLASSES OF COLORS SHOULD BE PERMITTED TO BE USED.

1. All colors, irrespective of their class, whether animal, vegetable, or synthetic, which have been physiologically and chemically examined, and which will neither retard digestion nor have special physiological effects when consumed in quantities corresponding to two grains per day per adult (p. 106).

2. On the use of colors we recommend that any kind of a harmless color should be permitted provided it is not a color generally known to be poisonous, or generally found to be poisonous, or one that may be almost impossible to be produced without containing some poison within itself, when finished and ready for use (p. 119).

3. Only such colors as are guaranteed to be harmless by reliable manufacturers should be used in the manufacture of confectionery (p. 555).

WHY COAL-TAR COLORS SHOULD NOT BE BARRED.

4. Coal-tar colors, as a class, should not be prohibited; but all those coal-tar colors generally found to be poisonous, or which are hard to produce without containing poisonous properties when ready for use, should be forbidden the privilege of being used or offered for sale for use in food (pp. 116, 117).

5. I ought to put in a plea for the use of coal-tar colors, harmless, of course, for the reason that we have not as yet been able to find any vegetable coloring that is suitable that will give us the results that we require. Coal-tar colors, as everybody knows, are much stronger and are more soluble and not acted upon by acids, whereas the vegetable colors, with but one exception, which is a dark red, we have found great difficulty in making use of for bottled soda water. Almost all the vegetable colors either fade out or change on account of the citric acid in the syrup or food, or form in a little while a precipitation which renders the goods unsalable (pp. 119, 120).

6. * * * aniline butter colorings * * * are superior to all vegetable colors in the following points: (1) Shade and brilliancy. (2) Strength, by which less foreign material is introduced into the butter. (3) Permanency when exposed to light and cold storage. (4) No effect on the taste or flavor. (5) A clear solution

without sediment or mud which gives the butter a uniform tint without specks (pp. 176, 177).

7. We recommend * * * that the use of harmless coal-tar colors, such as chrysoidine, tropæoline, azoflavine, rocelline, ponceau, Bordeaux, Biebrich red, sulphonated fuchsin and naphthol yellow S be allowed, subject to declaration on the label of the quality and quantity of the color used (p. 226).

RELATION OF LABEL TO COLORED FOOD PRODUCTS.

8. * * * if it seems wise in the opinion of the commission to allow certain coloring matters in food products, then the names of the coloring matter ought to be stated on the article (p. 109).

9. * * * I suggest that the use of aniline colors should be made proper if it is so stated on the label (p. 127).

10. We recommend * * * that the use of harmless coal-tar colors * * * be allowed subject to declaration on the label of the quality and quantity of the color used (p. 226).

LABELING OF FOOD COLORS AS DISTINGUISHED FROM COLORS FOR OTHER USES.

11. We recommend that if by any means the Government has the power under the food laws to compel color manufacturers so to do, they be compelled to label all packages containing colors intended to be used in articles of food as colors intended for such purposes as distinguished from colors intended for other purposes; * * * (pp. 555, 555*a*).

THE TEST OF A HARMLESS COLOR.

12. A harmless color is one "which will neither retard digestion nor have special physiological effects when consumed in quantities corresponding to two grains per day per adult" (p. 106).

QUANTITY OF COLOR TO BE CONSIDERED IN DETERMINING HARMLESSNESS.

13. The quantity of 2 grains is mentioned here because in confectionery where these harmless colors are more used perhaps than in any other product, it would be a proportion of one part of color to 3,500 parts, representing 1 pound of color to 3,500 pounds of confectionery, and that is why that was accepted, because that is practically the maximum quantity used in confectionery (p. 106).

14. In the very minute quantities in which the colors are used in carbonated beverages, it would seem a great hardship to prevent us from using coal-tar colors. One ounce of coal-tar red will color satisfactorily from 1,000 to 2,000 gallons of soda water. Of yellow and orange 1 ounce will color from 1,000 to 8,000 gallons. It is readily seen that unless the coloring used is absolutely a violent poison it can have absolutely no effect on the consumer who takes it in an 8-ounce glass, and who could not possibly consume half a gallon or a gallon of that product (p. 119).

15. On this basis 1,000 pounds of butter would contain 420 grains of aniline color (p. 183).

16. One ounce of color to 30 pounds colored food (p. 135).

THE NUMBER OF DIFFERENT COAL-TAR COLORS REQUIRED.

17. And if I were on this committee I would advocate taking out two or three dyestuffs which beyond any shadow of doubt are harmless, and which have been experimented upon, and which would be sufficient for all the purposes of the industry * * * none of these colors are patented, anybody can manufacture them (pp. 147, 148).

18. You have been told that the food commission of the State of Pennsylvania is going to rule that seven colors shall be allowed in the State of Pennsylvania. That is not enough, because it is not possible to reproduce all of the required shades with those colors unless you take the seven primary colors, when you can reproduce any colors. But it is absolutely necessary to have about three yellows to meet the requirements of the trade. Some of the articles that are put out have an organic acid nature, and the anilines will stand that. * * * On that account the character of the food product must be taken into account in the use of the color, and the confectioners have about 30 colors that I know positively of * * * (p. 160).

19. We recommend that * * * chrysoidine, tropaeoline, azoflavine, rocelline, ponceau, Bordeaux, Biebrich red, sulphonated fuchsin, naphthol yellow S be allowed * * * (p. 226).

MANUFACTURING REQUIREMENTS WHICH COAL-TAR COLORS MUST FULFIL.

20. Coal-tar colors, as everybody knows, are much stronger and are more soluble and are not acted on by acids. * * * Almost all the vegetable colors either fade out or change on account of the citric acid in the syrup or food, or form in a little while a precipitation which renders the goods unsalable (p. 119).

21. There are many of these colors (coal-tar colors) that will not stand the natural acids produced in manufacturing foods. For instance, in making confectionery, in boiling candy you make a certain grade of candy where the mixture is boiled to 230°. A certain color will stand that temperature. Then you take another candy and that is boiled to 320° or 340° F., and the colors that will stand 230° will in many cases not stand the temperature of 340°, while another class of colors will stand that temperature. So you have got to distinguish and get a color that will stand these difficult requirements (pp. 159, 160).

GUARANTEES AND GOVERNMENT CONTROL.

22. Only such colors as are guaranteed to be harmless by reliable manufacturers should be used in the manufacture of confectionery (p. 555).

23. We recommend * * * that the Government procure samples of such colors from time to time, wherever they have jurisdiction so to do, and if such colors be not legal for such purposes under the food law, that the same be prosecuted and driven off the market in so far as the Government has the power to do so; and if no means can be devised to compel such labeling of colors intended for food purposes, then that the Government procure such evidence as possible as to the purpose for which a color is intended to be used, and if such evidence shows a food purpose, that such color be prosecuted if illegal under the food law (pp. 555, 555 a).

24. See page 13.

25. The chemical test is the first, and that might throw out a color on account of its containing a little tin or zinc, or some substance foreign to the food product. Whether that is deleterious in the quantity in which it is present or not is immaterial (p. 160).

26. In a brief filed the following suggestions were made:

It must be stated that all the chemist can determine is whether or not the colors contain some impurities that are known to be of poisonous nature. Aniline colors are or can be made entirely free of such impurities, and with this fact established the task with the chemist is exhausted. When it comes to decide the question whether or not a color by itself, when free of all impurities, is injurious to health or not, then the chemist is not the proper authority; it is for the physiologist and for the medical profession to pass on such questions. Chemical theories go for nothing in deciding such questions. It would not even do to classify colors or other substances according to their makeup, as it has been shown again and again that substances belonging to the same chemical class are entirely different in regard to the physiological conduct.

Reliable information on this subject can be gained only by physiological experiment, as we can not say definitely whether a substance, color or any other, is injurious or not without finding out for every substance by experiment. This has been done for a considerable number of aniline colors, and these experiments are the only things that deserve any attention. Everything else is idle talk. * * * There are a very large number of aniline colors that have not been treated yet, and we are safe in saying that among these will be also some harmless and others injurious. As they have not been experimentally tested, we do not know which are harmless and which are not; It will therefore be clear that a law forbidding the indiscriminate use of aniline color for the purpose of coloring articles of food is necessary and useful.

But if the meaning of the law is to prevent only the use of injurious colors, as it appears to be, then the way to proceed would be very definitely outlined. Besides physiological colors, all such colors should be forbidden that have been found to be injurious and such aniline colors as have not been tested sufficiently. There will then be left over a number of aniline colors which have been proven by experiment to be entirely harmless, even if taken in large doses.

The experimentors were quite impartial. They had no preconceived ideas, but started simply to find out the true state of affairs. Their reports are therefore very reliable, and it will not do to overlook or to ignore them. The colors that they found harmless can be considered perfectly safe, so much the more as the doses conveniently taken with food would be much smaller than the doses that have proven to be harmless. These harmless aniline colors carry all the shades wanted in the food industry. The law should provide that one of these colors (or mixtures of these) must be used when a food article is being colored, because these few aniline colors are the only colors that can be considered perfectly safe as far as our present knowledge goes. Nothing should be left to guesswork or experimenting, as is the case just now. The colors that are permitted should be enumerated by their scientific as well as by their commercial names, and only such colors should be listed as permissible for coloring food products as have been proven to be harmless, even in large doses. Provision should be made to insure the purity of the colors sold for coloring food; the manner of packing such colors and the labeling of same should be laid down clearly, and all colors now listed, aniline as well as physiological colors, should be strictly forbidden. If the problem is viewed without preoccupation and prejudice, the facts given above will speak for themselves.

VIII. INVESTIGATIONS, OTHER THAN ON ANIMALS, BEARING ON THE HARMFULNESS OF COAL-TAR COLORS.

PFEFFER.

Pfeffer, writing on the Absorption of Anilin Colors by Living Cells, summarizes his results as follows:

The relatively little poisonous Methylene Blue does damage protoplasm in a solution of 0.001 per cent.

Methyl Violet: This coloring matter is not only stored up in the juices of the cell, but is also able to color the living protoplasm, and care is necessary, on account of the poisonous nature of the Methyl Violet, to prevent damage; these cautions are based upon solutions of 0.0003 to 0.00001 per cent strength.

Methyl Violet less poisonous than Cyanin.

Bismarck Brown about as poisonous as Methylene Blue.

Fuchsin as poisonous as Methylene Blue.

Safranin as poisonous as Methylene Blue.

Methyl Orange is poisonous only to a slight degree.

Tropæolin 000, Tropæolin 00, and Rosolic Acid are not poisonous.

Methylene Green as poisonous as Methyl Violet.

Nigrosin as poisonous as Methyl Violet.

Eosin (Tetraiodo fluorescein) kills in 0.1 per cent solution, but lets live 24 hours in 0.01 per cent solution. (*Untersuchungen aus dem Botanischen Institut zu Tuebingen, 1886–88, vol. 2, pp. 179–331.*)

WINOGRADOW.

Winogradow reports on the influence of certain coal-tar colors on digestion, which experiments were carried out in glass. The conclusions arrived at are as follows:[1]

The twelve colors, Safranin (584), Azo Fuchsin G. (93), Coerulein S. (527), Jodeosin (516), Magdala Red (614), Benzopurpurin (277, 278, 279, 307), Ponceau 2R (55), Orange II (86), Phloxin RBN (?), Chrysanilin (532), Azoflavin (92), and Cerise (mixture of 448 and 532), even in amounts of a few milligrams, which in relation to the digestive fluid make up only a few tenths or hundredths of a per cent, exercise a strongly retarding, almost completely inhibitive, action upon the peptic digestion of albumen.

The thirteen colors, Chinolin Yellow (667), Acid Green (434, 435), Azo Acid Yellow (92), Naphthol Yellow (4), Primulin (659), Anilin Orange (87), Metanil Yellow (95), Methylene Green (651), Iodin Green (459), Yellow T (84), Anilin Green (?) Auramin O (425), and Martius Yellow (3), retard the digestive action noticeably, although to a slighter degree than the first 12 colors; in any event they are not indifferent. (*Zts. Nahr. Genussm., 1903, v. 6, p. 589.*)

HEIDENHAIN.

Heidenhain, in his book entitled "Ueber chemische Umsetzungen zwischen Eiweisskorpern und Anilinfarben" (Bonn, Germany, 1902), reports on the behavior of 70 different coal-tar colors, 3 intermediate products, and 4 raw materials toward various albuminoids such as serumalbumen, albumen, and casein.

Of these 70 colors, 21 have been investigated physiologically, and the results embodied in this report; and of these 21, 17 were on the United States market as food colors and 4 were not on this market.

Limiting the attention to the 17 that were offered, 4 of them are among the 7 permitted colors of Food Inspection Decision 76.

In the cases where albumen and casein were used, they were employed in 0.5, 0.1, 0.02, 0.01, and 0.005 per cent solutions, acidified with acetic acid; the coloring matter was employed in a 1 per cent solution in the first strength, in 0.1 per cent solution in the second and third strengths, and in a 0.02 per cent solution in the fourth and fifth strengths, and one volume of coloring-matter solution was brought in contact with five volumes of albuminoid solution.

The following colors precipitated the albuminoid in all the strengths. The numbers in parentheses indicate the Green Table numbers;

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[1] It is not always possible to identify the trade names given by Winogradow with specific numbers in the Green Tables; the numbers in parentheses after the name indicate the number in the Green Tables wherever that identification could be made with any reasonable certainty; wherever two or more numbers appear, the context indicates that the name might apply to any one or all of them.

the numbers in italics are those of the permitted list of Food Inspection Decision 76: (55) Ponceau 2 R, (65) Fast Red B, (434) Light Green SF bluish, (*56*) Ponceau 3 R, and (106) New Coccin.

The following precipitated in all but the fifth strength: (*107*) Amaranth.

The following precipitated only the first three strengths: (14) Orange G and (*85*) Orange I.

The following precipitated only the first two strengths: (462) Acid Magenta.

For the basic colors the method of testing was different from that described for the acid colors and the amounts employed were not so definitely set forth. A 1 per cent solution of serum albuminoid was employed; the solution of coloring matter used varied in strength from 0.5 to 1 per cent (*b*), a "very dilute solution" (*a*) being also employed. The annotations given by the author (p. 114) are herewith reproduced in full:

(17) Chrysoidin Y. (*a*) In a very dilute solution the yellow base is at once liberated, and when sufficient color is added albumen precipitation takes place. No color change on heating. (*b*) Turns yellow at once. Further additional color produces a brown and albumen precipitation.

(201) Manchester Brown. (*a*) Becomes yellow at once, due to separation of the free base. On heating no change. (*b*) Becomes a discolored brown, and produces a nice brown albumen precipitate.

(425) Auramin O. (*a*) No change. (*b*) Precipitates albumen strongly.

(427) Malachite Green. (The oxalate of the color was used.) (*a*) Color changes from a blue-green to a more pure green. (*b*) Cold, no precipitation of albumen; heated, sudden precipitation of albumen.

(428) Brilliant Green. (A sulphate of the color was used.) (*a*) Becomes milky. (*b*) Immediate precipitation of albumen.

(448) Magenta. (Acetate and nitrate were used, and in both acted the same.) (*a*) Color changes from yellowish-red to rose-red. (*b*) Albumen precipitation abundant, even in the cold.

(451) Crystal Violet. (*a*) The color loses its reddish cast and changes to pure blue. (*b*) Albumen precipitated by the use of much color.

(*4*) Naphthol Yellow S was tested as the free-color acid and not as the sodium or potassium salt, which is its commercial form. It precipitated the albuminoid from the following solutions: 1. 1 per cent serum albumen in water. 2. 0.5 per cent serum albumen in 10 per cent acetic acid. 3. Casein, 0.5 per cent in 10 per cent acetic acid. 4, Serum albumen; 5, Casein; 6, Nuclein, all in 0.5 per cent solution in 0.2 per cent sodium hydroxid. 7. Nucleinic acid in 0.5 per cent water solution.

The relationship between the amounts of color-acid and albuminoid solution here used does not appear definitely in the book.

Having reference now to the literature and the physiological action, as compiled herein, it will be observed that the ability to precipitate albumen, or not to precipitate it, under the conditions of Heidenhain, appears not to have any direct connection with the results obtained by actual physiological test on animals or man. For instance, among the five that precipitated all five strengths of albumen and casein, No. 65, Fast Red B, of the Green Tables, has been found to be not harmful by tests actually described; No. 56, Ponceau 3 R, belongs to a class of colors generically permitted by the law of Austria; Nos. 55, Ponceau 2 R, and 106, New Coccine, have been reported on both favorably and unfavorably by different experimenters; No. 434, Light Green SF bluish on physiological examination has been described as suspicious.

From this it appears that two colors, physiologically probably harmless, precipitated all the five strengths of albumen, and three colors, which are perhaps no more than suspicious, likewise precipitated all five strengths of albumen.

No. *107*, Amaranth, which has been examined with favorable results by two different experimenters, precipitates four out of the five strengths.

Of the two colors precipitating the first three strengths, both have been examined physiologically with favorable results, namely, Nos. 14, Orange G, and *85*, Orange I.

The color which precipitated only the first two strengths, namely, No. 462, Acid Magenta, has likewise been examined physiologically, and the reports are favorable.

Thus it would seem that there is no definite connection between the physiological action and the ability to precipitate albumen from acetic acid solution in the case of acid colors.

In the case of basic colors the situation seems to be somewhat different.

OTHER AUTHORS.

Rosenstiehl (*Fifth International Congress of Applied Chemistry, Vol. III, p. 700*) states that when the color is present in an excess, yeast absorbs 8 per cent of Magenta (448) and 5 per cent of Malachite Green (427, 428). The Acridins (528–538), the Thionins (649–657), the Safranins (583, 584), and the Rosanilins (447–448) dye yeast the best; solutions containing 3 per cent by weight of the dry weight of the yeast are completely decolorized by such yeast and at ordinary temperatures. The Eosins and Phthaleins dye the yeast only incompletely, whereas Azo dyes (7–393) (with the exception of Benzo Purpurin, 277, 278, 279, 309) do not dye yeast at all. Such dyed yeast, however, is not dead; it has merely lost its power to cause fermentation. The numbers in parentheses are the corresponding numbers in the Green Tables as nearly as they could be identified.

Bokorny (*Chem. Ztg., 1906, v. 30, p. 217*) examined Magenta (448), Safranin (584), Victoria Blue (487, 488 or 490), Methylene Blue (650), and Alizarin Blue (562 or 563) (the numbers in parentheses are the probable Green Table numbers) as to their effect on micro-organisms such as yeast cells, infusoria, and the like, and found that these dyes in a concentration of 1:100,000 killed them, whereas strychnin nitrate in the same concentration is substantially without effect. Death is caused by absorption of the dye by the albumen of the protoplasm. The dyes seem to be absorbed not only by living albumen, but also by living nerve cells and fibers. Pure anilin or coal-tar colors, however, are not poisonous in the ordinary meaning of the words, that is, humans are not likely to be easily injured by them.

Houghton (*J. Amer. Chem. Soc., 1907, v. 29, pp. 1351–1357*) shows that Bismarck Brown (197, 201) and Crocein Scarlet (160?) hinder the peptic digestion of fibrin, casein, and albumen.

Stilling (*Anilinfarbstoffe als Antiseptica, 1890, v. 2, pp. 55–56*) states that he found the animal cells to be affected by pure coal-tar colors in the same way that vegetable cells were affected.

Penzoldt, based upon the experimental work of Beckh (*Archiv. Exp. Path. Pharmak. 1890, v. 26, p. 310*), reports as follows:

Of the 15 dyes—

	Name.	Green Table number.
1.	Malachite Green	427
2.	Hofman's Violet (methyl variety)	450
3.	Methyl Violet	451
4.	Rose Bengal (Erythrosin)	*517*
5.	Phenyl Blue	480
6.	Methylene Blue	650
7.	Fuchsin	448
8.	Coralline	484
9.	Eosin	512
10.	Methyl Orange	87
11.	Vesuvin	197
12.	Tropaeolin	88
13.	Scarlet Red	(?)
14.	Congo Red	240
15.	Indulin sulpho acid	601

all of which are water soluble and when used were free from arsenic, only the first six in saturated solution arrested the development of *Staphylococus pyogenes aureus*, and of these six all but the Erythrosin and Methylene Blue arrested the growth of anthrax bacillus.

Of these six when injected into rabbits subcutaneously the following produced no changes of consequence: Erythrosin (250), Phenyl Blue (100), Methylene Blue (75). The numbers in parentheses show the number of milligrams of dye per kilo body weight of the rabbits.

Methyl Violet (50) produced only local changes, such as gangrene.

Malachite Green (100) and Hofman's Violet (20) produced muscular paralysis, which in the case of Malachite Green resulted fatally on the ninth day; in the case of Hofman's Violet the paralysis was complete on the tenth day.

The remaining nine colors are apparently of no effect upon staphylococus or upon anthrax.

H. W. Williams (*A Manual of Bacteriology, 1906, p. 200*), under "Disinfectants and Germicides," says:

> Aniline dyes. Many of these substances, notably pyoktanin (Methyl Violet), possess germicidal properties. A solution of 1:5000 will kill the anthrax bacillus in two

hours. A much stronger solution, 1:150, is required to kill the typhoid bacillus in the same time. Malachite Green is said to possess even greater germicidal power than pyoktanin. Methylene Blue also possesses considerable germicidal power.

IX. COMPILATION UNDER THE GREEN TABLE NUMBERS OF ALL INFORMATION AVAILABLE AS TO THE SUITABILITY OF COAL-TAR COLORS FOR FOOD.

GENERAL STATEMENTS.

Before entering upon a detailed study of what has been published for and against specific coal-tar colors, it is probably well to consider, for whatever they may be worth, some of the general statements that have been made, from time to time, in the literature relative to coal-tar colors, considered either as a whole or as subdivisions or classes thereof, and their physiological action or their suitability for use in foods.

1. Schultz (*Die Chemie des Steinkohlentheers, Brunswick, 1887–1890, Vol. II, p. 35*), after discussing the regulations of the German Empire in respect to food coloring, says:

> With respect to these regulations the artificial organic coloring matters can be regarded, in general, as harmless. For the purposes of dyeing magenta made by means of arsenic acid, further picric acid, and those coloring matters which occur as oxalates or zinc chlorid double salts, such as Methylene Blue and Bitter Almond Oil Green, can be used. The use of the substances named for the coloring of food products is, however, suspicious and should not be permitted.

2. Stilling (*Anilinfarbstoffe als Antiseptica, Strassburg, 1890, Vol. II, pp. 55–56*) says:

> In view of the fact that the most innocent substance, such as distilled water or common salt, when introduced in large quantities into the organism can act fatally, the anilin coloring matters therefore, particularly if they be free from all admixtures, such as arsenic, copper, and chlorid of zinc, are to be regarded as wholly nonpoisonous.
>
> All experience gathered since my first publication has likewise fully confirmed this nonpoisonous nature.

3. Stilling (*Ber. Klin. Wochensch. 1890, p. 531*) also says:

> Proceeding from purely theoretical views, and based upon these botanical and physiological experiments, I have recommended anilin colors as antiseptics for the following reasons:
>
> 1. They are to be designated as absolutely nonpoisonous. This will be confirmed by every chemist acquainted with the relevant details, and also from the medical side this has long ago been determined by Grandhomme. The publications of this author, who made extensive observations and experiments in the anilin factory of Meister, Lucius & Bruening, appeared in the beginning of the eighties, and has hardly become known in medical circles. However, I was first made acquainted with this by my colleague, Prof. Rose, in Strasburg.
>
> 2. That it is possible to bring about death in experimental animals by introducing large amounts of the coloring matter into the peritoneal cavity proves nothing against

the nonpoisonous nature of these substances. This action is to be regarded as a purely mechanical one, a view which I will thoroughly confirm in my second communication.

With respect to the anilin colors not soluble in water, Ehrlich long before me, in his excellent publication on the oxygen requirements of the organism, has arrived at this view, and has excellently described it as merely a penetration of the organs.

4. Lehmann (*Methoden der Praktischen Hygiene, Wiesbaden, 1890, p. 543*) says:

The hygienic significance of coal-tar colors has heretofore been judged quite variously. When the intensely poisonous nature of the first impure and particularly arsenic-containing coloring matters became known the inclination was to judge the coal-tar coloring matters very strictly; when it was subsequently recognized that the contaminations were largely the cause of the harmfulness to health, there followed a period in which no poisonous coal-tar coloring matter was known in any pure condition. (Eulenberg & Vohl, 1870.) More recent investigations, however, have disclosed a series of coal-tar coloring matters which, as a matter of fact, possess a considerable poisonous action, and already cases, although not numerous, have become known in which serious and even fatal poisonings by means of pure coal-tar colors have arisen. Alongside of this there still continue to exist the possibilities described by Eulenberg and Vohl (Viertel-Jahressch. für Gerichtliche Mediz, 1870), whereby harmless coloring matters become harmful; but the realization of these possibilities has become essentially more seldom through improvements and changes in manufacture.

5. Stilling (*Arch. Exper. Pathol. Pharmak., 1891, v. 28, p. 352*), in speaking of the anilin colors as antiseptics, says:

It is the nonpoisonous nature of these substances, their easy solubility and diffusibility, and above all their inability to coagulate albumen which lends them their importance, which now can be only difficultly denied.

NOTE.—The work of Heidenhain abstracted in Section VIII does not fully bear out this article.

6. Erdmann (*Pharm. Centralh., 1892, v. 33, p. 357*) says:

The sulphonated as well as the carboxylated coal-tar dyes will not have any pronounced action on the organism. Acid dyes may therefore be regarded in general as nonpoisonous, whereas in the case of basic dyes a physiological examination is to be recommended before they are permitted to be applied to articles in daily use or indeed to be used in food or drink.

NOTE.—Out of the 80 different dyes on the food-color market in the summer of 1907 whose composition was avowed, 15 were basic and 65 were acid.

7. Tschirch expressed himself as follows:

1. The coal-tar colors, and in a narrower sense the anilin colors, are no longer harmful on account of their arsenic content, since at the present time the great majority of them are prepared free from arsenic.
2. Some colors have shown themselves to be harmful to the system.
3. Coal-tar colors, in general, should therefore be permitted for the coloring of foods, but those that have been found to be harmful should be expressly and specifically forbidden.

4. The amount of coloring matter which has been determined quantitatively in bonbons and liqueurs is so small that even the ones regarded as poisonous would not be able to develop their harmful effects. (*Zts. Nahrs. Unters. Hygiene Waarenk., 1893, v. 7, p. 338.*)

8. Georgievics (*Lehrbuch der Farbenchemie, Leipzig, 1895 p. 10*) says under "Poisonous nature of the coloring matters:"

It is a little known fact that of the very large number of organic coloring matters only a few have been found to be poisonous; these are Picric Acid, Victoria Orange (Saffron-surrogate), Aurantia, Metanil Yellow, Orange II, and Safranin. The prejudice which is still quite widely accepted that most of the artificial coloring matters are poisonous dates from the early periods of anilin-color manufacture, at which time magenta and the coloring matters made from it occurred in commerce highly contaminated with arsenic. At present, however, these coloring matters are prepared absolutely free from arsenic, and are, as such, nonpoisonous. A few coloring matters which occur commercially as zinc chlorid double salts, such as Methylene Blue and Malachite Green, may be harmful in consequence of their zinc content, and should therefore never be employed in the coloring of food products. * * * In consequence of their physiological activity some coloring matters are employed as medicines, indeed principally Methyl Violet, Auramin and Methylene Blue. The first two, known as blue and yellow Pyoctanin (pus-destroying) are, owing to their great antiseptic action and diffusibility, valuable medicines; on account of the unpleasant coloring effect accompanying them they are but little used. Methyl Violet was first recommended as an antibacterial remedy in diseases of the eye by Stilling; subsequently it has been employed in other special cases; its principal use, however, is in surgery for the prevention of malignant proud flesh.

The use of Auramin is entirely analogous.

Methylene Blue (as a free base) is used principally as an analgesic (pain-relieving remedy) and is given internally; on account of its ability rapidly to diffuse through the tissues of the nervous system; it can also be introduced by injection. It is used as a remedy against malaria, carcinoma, Bright's disease, etc.

The following have been tested as remedies or as antiseptics: Safranin, Lydin (Mauvein), Vesuvin, Anilin Blue, Carbolic Magenta, Alizarin Yellow C (Gallacetophenone), etc. The potassium salt of Dinitro-ortho-cresol was brought into commerce by the Farbenfabriken vorm. Friedr. Bayer & Co., under the name of Antinonnin, and has given excellent results as a means against formation of mold in cellars and against wood fungi.

9. Weyl (*Handbuch der Hygiene, 1896, p. 378*) says:

A few organic coloring matters, but only a very few, possess poisonous properties. A rule by means of which the poisonous or nonpoisonous nature of organic coloring materials can be determined without experiment is unknown even for those coloring matters whose constitution has been determined and experiments on the poisonous nature of organic coloring matters are very few in number.

10. Lewin (*Lehrbuch der Toxicologie, 1897, p. 230*) says:

In the use of various fabrics or of foodstuffs, which are colored with anilin or coal-tar colors, or in commercial contact with such colors, local and general symptoms of poisoning, such as eczema, swelling of the face, vomiting, diarrhea, anæsthesia, paresis, etc., have been observed. These are generally due to the toxic nature of the coloring matters, frequently to harmful ingredients of the same, and hardly ever to poisonous mordants. Many workmen in anilin factories show permanent spots; for example, on the cornea and conjunctiva, head, chest, face, and neck, without any interference with their general condition. Local changes of more serious nature have more frequently been observed in the mucous membranes and on the skin. Thus, in one case, a camel's-hair pencil, soaked with anilin color, accidently entered the eye, and at first nothing was noticed but a violet blue coloration, later inflammaion and chemosis took place. I have observed local swelling and indurations of the skin, particularly on the cheeks, in the case of children after they had worn caps colored with anilin dye.

11. Winton (*Connecticut Agric. Exper. Sta. Report, 1901, p. 181*) says:

Although there is evidence that most of the coal-tar dyes are not injurious to some of the lower animals, it is not safe to assume that they are entirely harmless to human beings. The dog, an animal used in most of Weyl's experiments, has a proverbially strong stomach, and eats with no apparent discomfort many things which would disturb the digestion of a man.

12. Chlopin, in his book published in 1903 (see p. 75), states as follows:

(*Page 114.*) * * * All the dyes examined by me I divided into three categories: Dyes which caused striking general symptoms of poisoning and led to the death of the animal, or would have led to it if the experiments were not purposely discontinued, I designate by the term poisonous; dyes which induced some separate and temporary symptoms of disease, for instance, vomiting, diarrhea, separation of albumen in the urine, the general condition remaining normal, I designate as suspicious; lastly, the dyes which caused no apparent disturbance during the experiments are designated by the term nonpoisonous. I purposely do not call the last category harmless, because by our experiments the question could not be decided negatively as to whether the nonpoisonous dyes did not cause some finer pathological changes in the organism and functions which could not be detected by simple observation.

(*Pages 219–221.*) Thus, according to all the investigators quoted, there were found altogether 22 poisonous and harmful dyes, out of about 60 dyes examined; which makes 36.7 per cent of poisonous and harmful dyes among those examined.

My investigations gave 30 per cent of poisonous and 40 per cent of suspicious dyes.

The percentages above given have a fairly well established basis, since they were obtained by the examination of 100 dyes, which is about one-fifth of all the dyes in commerce. Further, considering the distribution of the poisonous and harmful dyes according to various chemical groups, we find that they occur in 12 of the 18 groups, and we can not note any regularity in this distribution; it is impossible to say that there is any definite connection between the fact that the dye belongs to a certain chemical group and its action on the animal organism. Usually among the dyes of one and the same group there are some harmful ones, but there are also some harmless ones, and the ones and the others have very similar composition. This or that action of the dyes on the animal organism, as we shall presently see, is determined more by the delicate difference in the internal structure of their molecules than by those differences on which is based, at the present time, the classification of the aromatic dyes.

On the basis of the whole experimental material on hand—mine and that of other investigators—I can make only the following very few and purely empirical generalizations:

1. According to the shade produced, the poisonous and harmful colors are distributed as follows: Most of all poisonous dyes are found among the Yellows and the Oranges; then come the Blues, then the Browns and the Blacks; there are very few harmful dyes among the Violets and Greens; among the Reds was found only one suspicious one, and no poisonous ones.

2. The most poisonous dyes belong to the Nitro, Azo, Triphenyl, and Thiazin groups, and also to the Auramins.

3. A whole group of poisonous and harmful dyes is formed by the new sulphid dyes known as Vidal's dyes.

(*Page 224.*) By the facts and observations quoted above is corroborated the opinion that in general the coal-tar dyes, according to the composition and properties, appear as substances foreign to the animal organism, and may influence harmfully the vital functions, even in those cases when they do not possess distinctly poisonous properties. For this reason many hygienists make it a principle not to allow the coloring of food products or of beverages with coal-tar dyes, independently of the fact whether they prove in actual experiments on animals poisonous or not.

We must therefore agree with M. Rudner that food of the masses require the most far-reaching protection, maintaining them free from foreign additions.

13. Koenig (*Die Menschlichen Nahrungs- und Genussmittel, Berlin, 1904, Vol. II, p. 462*) says:

Even though the majority of the anilin coloring matters, in view of the small amounts in which they are generally employed, can not be regarded as directly harmful to health, yet the objections to their use in the coloring of food products for the purpose of substituting or strengthening a natural color lies in the deception connected therewith * * *.

14. Fraenkel (*Arzneimittel-Synthese, Berlin, 1906, p. 570*) says:

It is clear that the coloring property of these chemical substances stands in no relation to their physiological actions, but, on the contrary, the physiological actions depend only upon the general structure of these substances, and therefore upon their membership in definite chemical groups.

(*Pages 574–5.*) We see, even in considering this group of substances, that they do not possess any specific action, but they are capable of use, preferably by external application, as antiseptic materials, as materials which in their action stand somewhere between carbolic acid and corrosive sublimate, and whose coloring property, in consequence of which they were primarily selected, is directly a hindrance in this use, since the coloring of the bandages and the hands of the operators and the skin of the patients certainly can not be regarded as a pleasant occurrence; that the antiseptic action stands in some relationship to the properties of the substances as coloring matter must be positively contradicted. It depends only on the general structure of the substance, and does not stand in any direct relation to the chromophore and auxochrome groups of the substances, but more closely to the aromatic nucleus. Indeed, it may happen that an auxochrome group diminishes the antiseptic activity of such a substance.

NOTE.—The dyes referred to belong to the Monazo, Disazo, Triphenyl-methane, Xanthin, Azin, and Thiazin classes.

(*Page 92.*) The investigations of Ehrlich have shown that basic dyes color the brain gray, and, moreover, they color nerve fiber very well, and are therefore to be regarded as neurotropes. The dye acids, on the other hand, do not dye nerve fiber, and in particular the substituted sulphonic acids do not dye tissue at all.

15. Meyer, in his paper on "A preliminary communication on the toxicity of some aniline dyestuffs" (*J. Amer. Chem. Soc., 1907, v. 29,* p. 892), says:

(*Page 893.*) "A manufacturing confectioner of this city, for whom I made examination of colors used by him, informs me that a yellow color sold as Auramin has such high tinctorial power that 1 ounce will color 2,000 pounds of candy to the highest yellow tint required in his business. It is obvious that the toxicity of such a body would have to be very high to render it harmful in such use." Conclusions of this kind do not take into account the possible detrimental action ensuing on healthy as well as diseased persons from long-continued use of small quantities of foreign substances.

(*Page 909.*) The same author raises objection to feeding experiments on the ground that substances are thereby introduced greatly in excess of the amounts generally found in foods and that the ill effects "are liable to be due to the excess and in long-continued experiments due to a cumulative action of the excess." Surely if excessive amounts have a cumulative action, small amounts may also finally show toxic effects due to retention and accumulation of the poison. To declare a substance entirely innocuous would require evidence as to its nontoxicity both to normal and diseased persons after its long-continued administration in both small and large doses. The most extreme contingencies would have to be provided for. The above objections to feeding experiments are therefore not valid. It is hoped that a study of the effects on metabolism of some of these substances will help to further elucidate the subject.

He summarizes the results of his physiological investigations of seven different coal-tar colors as follows:

1. Several commercial organic dyestuffs (Curcumin S, Tartrazin, Naphthol Red S, Carmois in B, Naphthol Yellow S, Gold Orange, and Ponceau 2 R) were studied as to their general effects on dogs when administered in varying amounts and during fairly long periods (two weeks).
2. None of these dyestuffs under the conditions above indicated exhibited any marked degree of toxicity. There was only one fatal result, which may have been due to influence independent of the action of the colorant.

Similar quotations from the literature could be added to the foregoing, but these are beyond question sufficient to show that a wide divergence of opinion as to the harmless or harmful nature of the coal-tar colors as a class does exist among scientific men, and that all those above quoted agree that there are some at least of the coal-tar colors which even in a pure state may be harmful to human health, and that the question of actual harmfulness under the conditions of actual use in foods and the consumption of foods is regarded by some as being properly answered in the negative and by others as being properly answered in the affirmative. The question of amount of color employed in the food products and the amounts of such food normally eaten are therefore raised by some as the deciding factors.

In this connection the following statement from page 49 of the arguments before the Committee on Patents in the House of Representatives, April 8, 15, 16, 22, 29, 1908, may be of interest:

> * * * It should be remembered that after a new chemical has been discovered and patented it requires as many as three years of experiment before we dare offer it in this country as a medicine for human beings. These experiments are conducted abroad before we receive it here. It is first tried on animals and gradually, with great caution, extended to human beings in the foreign hospitals, so as to ascertain its physiological effects quantitatively upon the various organs, both when those organs are in the healthy state and when they are affected by various disorders * * *.

A search of the literature herein compiled fails to disclose any such searching physiological examination of any of the coal-tar colors recommended for use by human beings in food products, as is asserted in the above quotation to be necessary in the case of a new chemical intended for use as a drug. If Fraenkel, as quoted on page 60, is correct in his statement that coal-tar colors act physiologically because they are chemicals and not because they are coloring matters, then coal-tar coloring matters prior to use in foods, in which they are used by the young and the old, the well and the sick, without restriction and without supervision, should also be thoroughly tested, and very few, if any, coal-tar colors seem to have been examined with the thoroughness set forth in the above quotation. That uniformity and purity of product is necessary in order to be sure that the chemical is going to act physiologically in the same way every time is obvious. According to Fraenkel, what is true of a chemical is just as true of a coal-tar color, and if uniformity of strength and cleanliness of product are desirable when a chemical is to be used as a medicine, such properties are equally desirable when a chemical is to be used as an ingredient in food.

CLASSIFICATION OF OPINIONS IN LITERATURE AND IN LEGAL ENACTMENTS SHOWING CONDITION OF THE MARKET IN 1907.

The literature and legal enactments hereinafter grouped under the relevant Green Table numbers have been classified as (1) unfavorable—i. e., only unfavorable reports found in the literature; (2) favorable, and (3) contradictory reports, as each case required; so that under each Green Table entry there is not only the relevant literature, but also the character assigned to it for the purpose of coming to a conclusion as to the propriety of the use of such color in foods as based on such literature, which conclusion formed in that respect the basis for Food Inspection Decisions Nos. 76, 77, and 106.

However, it does not follow that all dyes placed in the "favorable" list are actually harmless; the investigations or opinions reported of each may very well be based upon insufficient data. This classification, therefore, is merely intended to reflect the present state of the literature with respect thereto, and is not necessarily final nor conclusive.

In substantially all the recorded cases the observers directly or indirectly assert the absence of arsenic and mineral poisons in the dyes subjected to physiological test, but the kind of other impurity, if any, is not stated.

For the purpose of a comprehensive survey of the literature and legal enactments, the following tabulation is presented:

Condition of the United States color market in the summer of 1907.

[x=not on market. Figures indicate number of dealers offering sample. Cross lines separate the several groups.]

Green Table number.	Unfavorable.	Favorable.	Contradictory.
1	x		
2	x		
3	x		
[1]*4*		10	
5		x	
6	x		
8			5
9			1
11			2
13			6
14			2
15			x
16			x
17			2
18			1
28		x	
41			x
43			x
55			2
65		2	
70			x
78	x		
84			2
85		2	
86			8
87			x
88			x
89		1	
92		x	
93		x	
94	6		
95			2
97	1		
102		x	
103		6	
105		1	
106			5
107		7	
138			x
160			x
163			x
164	x		
166		x	
169		1	
188			1
197			4
201	2		
240		1	
269			1
277	x		
287			1
394		x	
398			2
399		x	
425	3		
427			2
428			3
433		1	
434	1		
435		4	
448			4
450			x
451			5
457			x
459	x		
462		2	
467		x	
477		x	
478	x		
479	x		
480			1
483			x
488, 490	x		
502	2		
504			5
512		3	
516	1		
517		5	
520		2	
521		x	
527		x	
530	x		
532	x		
563			x
572	x		
574	x		
576		x	
584			1
593		x	
599		x	
600		x	
601			1
602	x		
614	x		
620	x		
639	x		
649	x		
650			2
651	x		
654	x		
659	x		
667	1		
670	x		
675	x		
689			x
692		3	
Totals			
106	33	32	41
[2] 50	[2] 8	[2] 16	[2] 26

[1] Italicized figures are colors in the permitted list, Food Inspection Decision 76.
[2] On United States market in 1907.

This table shows that of the 106 coal-tar dyes examined physiologically only 50 were on the United States market; further, out of 33 "unfavorable" dyes 8, or one-fourth, were on the United States market; out of 32 "favorable" dyes 16, or one-half, were on the United States market, and finally that out of 41 "contradictory" dyes 26, or very nearly two-thirds, were on the United States market in the summer of 1907.

Assuming this classification to be substantially and essentially fair, the only Green Table numbers which are of interest for the present discussion are those classified under "favorable," because any color positively injurious or of doubtful character is considered as being properly excluded from use in food products. The Green numbers classified as "favorable" are 32 in number, as follows: *4;* 5; 28; 65; *85;* 89; 92; 93; 102; 103; 105; *107;* 166; 169; 240; 394; 399; 433; *435;* 462; 467; 477; 512; *517;* 520; 521; 527; 576; 593; 599; 600; *692.*

Of these 32, 16 were on the United States market in the summer of 1907 and their composition disclosed; they are presented in the following table, together with the number of dealers, out of a possible 12, offering them for sale.

Distribution of "favorable" colors on the American market in 1907.

Green Table number.	Number of dealers handling.	Green Table number.	Number of dealers handling.	Green Table number.	Number of dealers handling.	Green Table number.	Number of dealers handling.
4	10	103	6	240	1	512	3
65	2	105	1	433	1	*517*	5
85	2	*107*	7	*435*	4	520	2
89	1	169	1	462	2	*692*	3

From among these 16, six of the seven permitted colors of Food Inspection Decision No. 76 were selected. The process of selection and of elimination is described on page 166.

CLASSIFICATION ACCORDING TO CHEMICAL COMPOSITION AND SUITABILITY.

In the following table the chemical composition of the substances corresponding to the Green Table numbers is given, and their classification according as the literature is regarded as being (1) unfavorable, (2) favorable, or (3) contradictory in regard to the color. The colors are also arranged in the groups to which they belong chemically, so that this table shows: (1) The number of groups reported on in the literature, (2) the number and composition of members of each group so reported on, and (3) the interpretation here placed upon such reports in literature. This table is given in the expectation that it will be of use to chemists and physiologists.

Opinions as to suitability, classified according to groups and chemical composition.

(The chemical nomenclature is that of the Green Tables; "a" is used for alpha and "b" for beta.)

Unfavorable.	Favorable.	Contradictory.
NITRO COLORS.		
1. Symmetrical trinitrophenol.	4. Dinitro-a-naphthol-b-monosulphonic acid.	
2. Dinitro o and p-cresol.	5. Dinitro-a-naphthol-a- monosulphonic acid.	
3. Dinitro-a-naphthol.		
6. Hexanitro-diphenylamin.		
MONOAZO COLORS.		
78. Methyl-benzenyl-amido-thio-xylenol-azo-a-naphthol disulphonic acid.	28. p-nitro-benzene-azo-a-naphthylamin p-sulphonic acid.	8. Amido-azo-benzene-di- and monosulphonic acid.
94. Benzene-azo-pyrazalone-carboxy-disulphonic acid.	65. a-naphthalene-azo-b-naphthol-disulphonic acid.	9. Amido-azo-toluene-disulphonic acid.
97. Sulpho-o-toluene-azo-b-naphthol.	85. p-sulphobenzene-azo-a-naphthol.	11. Benzene-azo-b-naphthol.
	89. p-sulphobenzene-azo-di-phenylamin-sulphonic acid.	13. Benzene-azo-b- naphthol-b-sulphonic acid.
	92. Diphenylamin yellow with nitro-diphenylamin.	14. Benzene-azo-b-naphthol disulphonic acid G.

Opinions as to suitability, classified according to groups and chemical composition—Con.

Unfavorable.	Favorable.	Contradictory.
MONOAZO COLORS—continued.		
	93. p-sulphobenzene-azo-dioxy-naphthalene sulphonic acid. 102. p-sulpho-naphthalene-azo-b-anphthol. 103. p-sulpho-naphthalene-azo-a-naphthol-p-sulphonic acid. 105. p-sulpho-naphthalene-azo-b-naphthol monosulphonic acid. 107. p-sulpho-naphthalene-azo-b-naphthol-disulphonic acid.	15. Benzene-azo-b-naphthol disulphonic acid R. 16. Dimethyl-amido-azo-benzene. 17. Hydrochlorid of diamido-azo-benzene. 18. Hydrochlorid of benzene-azo-m-tolylene-diamin. 41. Hydrochlorid of toluene-azo-m-tolylene-diamin. 43. Toluene-azo-b-naphthol-sulphonic acid. 55. Xylene-azo-b-naphthol-disulphonic acid. 70. Dichloro-phenol-azo-b-naphthol. 84. p-sulphobenzene-azo-resorcinol. 86. p-sulphobenzene-azo-b-naphthol. 87. p-sulphobenzene-azo-dimethylanilin. 88. p-sulphobenzene-azo-diphenylamin. 95. m-sulphobenzene-azo-diphenylamin. 106. p-sulphonaphthalene-azo-b-naphthol-disulphonic acid.
DISAZO COLORS.		
164. Sulphobenzene-azo-sulphobenzene-azo-b-naphthol-sulphonic acid. 201. Hydrochlorid of toluene disazo-m-tolylene-diamin. 277. Ditolyl-disazo-binaphthionic acid.	166. Sulphobenzene-azo-sulphobenzene-azo-p-tolyl-b-naphthylamin. 169. Sulphotoluene-azo-toluene-azo-b-naphthol-a-sulphonic acid. 240. Diphenyl-disazo-binaphthionic acid.	138. Bisulphobenzene-disazo-a-naphthol. 160. Sulphobenzene-azo-benzene-azo-b-naphthol-monosulphonic acid. 163. Sulphobenzene-azo-sulphobenzene-azo-b-naphthol. 188. Disulpho-b-naphthalene-azo-a-naphthalene-azo-b-naphthol-disulphonic acid. 197. Hydrochlorid of benzene-disazo-phenylene-diamin. 269. Ditolyl-disazo-bi-salicylic acid. 287. Ditolyl-disazo-bi-a-naphthol-p-sulphonic acid.
NITROSO COLORS.	394. Dinitroso-resorcinol.	398. Nitroso-b-naphthol-b-monosulphonic acid.
STILBENE COLORS.	399. Azoxy-stilbene-di-sulphonic acid.	
DIPHENYLMETHANE COLORS.		
425. Hydrochlorid of imido-tetramethyl-diamido-diphenyl-methane.		
TRIPHENYLMETHANE COLORS.		
434. Dimethyl-dibenzyl-diamido-triphenyl-carbinol-trisulphonic acid. 459. Chlorid of heptamethyl-rosanilin chlorid. 478. Triphenyl-pararosanilin di- and trisulphonic acid. 479. Triphenyl-pararosanilin-trisulphonic acid. 488. 490. Hydrochlorid of phenyl-tetra- (penta) methyl-triamido-diphenyl-a-naphthyl carbinol.	433. Diethyl-dibenzyl-diamido-triphenyl carbinol-disulphonic acid. 435. Diethyl-dibenzyl-diamido-triphenyl-carbinol-trisulphonic acid. 462. Trisulphonic acid of rosanilin and pararosanilin. 477. Triphenyl-rosanilin-monosulphonic acid and triphenyl-pararosanilin-monosulphonic acid. 467. Disulphonic acid of dimethyl dibenzyl di-ethyl triamido triphenyl carbinol.	427. Chlorid of tetra-methyl di-p-amido-triphenyl-carbinol. 428. Sulphate or chlorid of tetra-ethyl-diamido-triphenyl-carbinol. 448. Hydrochlorid or acetate of pararosanilin and rosanilin. 450. Hydrochlorids or acetates of mono-di or trimethyl (or ethyl) rosanilins and pararosanilins. 451. Hydrochlorid of penta- and hexamethyl-pararosanilin. 457. Hydrochlorid, sulphate or acetate of triphenyl rosanilin and triphenyl pararosanilin. 480. Triphenyl-rosanilin and triphenyl-para-rosanilin sulphonic acids. 483. Aurin carbinol, oxydized aurin, methyl aurin, and pseudo rosolic acid.

Opinions as to suitability, classified according to groups and chemical composition—Con.

Unfavorable.	Favorable.	Contradictory.
XANTHENE COLORS.		
502. Triethylrhodamin.	512. Tetrabromofluorescein.	504. Hydrochlorid of diethyl-m-amido-phenol-phthalein.
516. Diiodo fluorescein.	517. Tetraiodofluorescein.	
	520. Tetraiododichlorofluorescein.	
	521. Tetrabromotetrachloro fluorescein.	
	527. Sodium bisulphite compound of cœrulein.	
ACRIDIN COLORS.		
530. Hydrochlorid of diamido-phenyl-dimethyl-acridin.		
532. Nitrate of chrysanilin and homologues.		
ANTHRACENE COLORS.		
		563. Dioxy-anthraquinone-b-quinolin.
INDOPHENOL COLORS.		
572. Tin compound of dimethyl p-amido phenyl-p-oxy-a-naphthylamin.	576. New Gray (composition unknown).	
574. Hydrochlorids of p-phenylene-diamin-p-amidophenol and diamido-diphenylamin.		
AZIN COLORS.		
602. Spirit Nigrosines.	593. Phenyl- and tolyl-safranins.	584. Diamidophenyl and tolyl-tolazonium chlorids.
614. Amido-naphthyl-naphthazonium chlorid and diamido naphthyl-naphthazonium chlorid.	599. Mixtures of dianilido-amido-trianilido-, and tetraanilido-phenyl-phenazonium chlorids.	601. Sulphonated indulins.
	600. Indulins and fluorindins.	
OXAZIN COLORS.		
620. Dimethylamido-dioxy-phenazoxonium carboxylate.		
639. Chlorid of dimethyl-amido-naphtho-phenoxazonium chlorid.		
THIAZIN COLORS.		
649. Zinc-double-chlorid of dimethyl-diamido-phenazthionium chlorid.		650. Chlorid of tetramethyl-diamido-phenazthionium.
651. Nitromethylene Blue.		
654. Chlorid of dimethyldiamidotoluphenazthionium.		
THIOBENZENYL COLORS.		
659. Dehydrothiotoluidin.		
QUINOLIN COLORS.		
667. Quinophthalone.		
SULPHID COLORS.		
670. Sulphohydro derivative of a polythiazin.		
675. Thiocatechin S (composition unknown).		
INDIGO COLORS.		
	692. Indigotin disulphonic acid.	689. Indigotin.

PHYSIOLOGICAL ACTION OF COAL-TAR DYES.

SUMMARY OF SYMPTOMS.

A rough summary of the symptoms noted or positively determined to be absent, the number of the deaths produced, and the number of cases in which nothing abnormal was noticed may serve as a convenient guide in considering the detailed statements hereinafter given relative to all the symptoms, clinical data, legislative and other publications, or permissions.

In the following tables are brought together most, if not all, of the recorded observations with respect to the humans and other animals upon which the physiological action of coal-tar dyes has been studied as well as the results of autopsies when recorded. The columns headed "Unfavorable," "Favorable," and "Contradictory" have the same significance as in the preceding table; italicized numbers are those of the permitted colors of Food Inspection Decision No. 76. Asterisked numbers indicate that the dye was administered hypodermically.

Experiments on Dogs.

Table I.—*Observations on dogs.*

(Reference to Green Table numbers.)

Symptoms.	Unfavorable.	Favorable.	Contradictory.
Deaths	1; 2; 3; 488 or 490; 574; 639 (2); 649.		16 (2); 55; 70*; 86; 87; 95 (2); 188*; 428 (2); 451 (2); 480*; 601; 650.
Autopsies	3; 488 or 490; 572; 574; 620; 639; 649.	103; 105; 399; 467; 477; 593; *692*.	16; 55; 70*; 87 (2); 45 (2); 457; 480 (2); 601; 650.
Stained skin	1		398.
Stained conjunctivæ	1		398.
Respiration difficulties	1; 3		
Temperature normal	2; 620; 649	467	451; 601.
Temperature high	488 or 490; 3		457.
Temperature low	488 or 490		451.
General depression	425; 649; 675		650.
Weakness	1; 3; 97; 649; 659		16; 483.
Loss of weight	1; 488 or 490; 639		17; 18; 41; 95; 138; 451; 650.
Emaciation	488 or 490; 639		138; 451.
Paralysis of heart			16; 87; 428.
Loss of appetite	2; 3; 97; 425; 530; 649; 659; 667; 670.	240; 394	16; 87; 138; 197; 428; 451; 483; 650.
Aversion to food	649; 3		451.
Colored urine	3; 6; 78; 94; 97; 277; 425; 434; 459; 478; 479; 502; 516; 530; 532; 614; 620; 651; 659; 667; 670.	*4*; 5; 28; 92; 93; 103; 105; 166; 240; 394; 399; 433; 521; 527; 576; 599.	8; 9; 11; 13; 14; 16; 55; 84; 86; 87; 88; 95; 138; 197; 269; 287; 398; 428; 448; 483; 504; 601; 650.
Colorless urine		240	287.
Urine not affected	488 or 490; 649	467; 477	451; 480; 601.
Albuminuria	1; 3; 78; 94; 97; 425; 434; 459; 479; 516; 580; 651; 659; 667.	*4*; 5; 28; 166; 240; 394	8; 9; 11; 17, 18, or 41; 70; 86; 88; 95; 188; 197; 269; 287.
Albuminuria doubtful	78; 478	5	138.
Alkaline urine	670	240	14; 88; 269; 287.
Bloody urine			451.
Phenol in urine			11; 88.
Kidney irritation	1		86.
Bladder irritation	639		650.
Thirst	3		16; 86; 87; 428.
Colored feces	94; 425; 488 or 490; 620	103; 105; 399; 477	87; 88; 398; 480; 601; 650.
Thin stool	425; 502; 614	527	16; 504.
Diarrhea	1; 3; 94; 97; 479; 425; 516; 614; 639; 649; 651; 670.	*4*; 92; 103; 105; 399; 527; 593.	11; 16; 84; 86; 87; 138; 269; 428; 483; 504; 584; 650.
Softened feces			95.
Bloody stool			650.

TABLE I.—*Observations on dogs*—Continued.

Symptoms.	Unfavorable.	Favorable.	Contradictory.
Pus in stool			650.
Stool desires	502		
Vomiting	1; 2; 3; 6; 97; 277; 425; 479; 488 or 490; 516; 532; 574; 614; 639; 649; 670; 675.	399; 433; 467; 576; *692*	11; 16; 86; 87; 95; 197; 269; 428; 448; 451; 483; 650.
Retching	675	28	16; 86.
Salivation	488 or 490; 639; 649; 675		584.
Anemia	488 or 490; 639		
Stupor			601.
Desire to sleep			451.
Catarrh of eyes and nose			451.
Cramps	1; 2		601.
Convulsions	2; 675		
Tremors			87.
Congestion	649		
Paralysis of limbs	1; 675		16; 55; 87; 428; 450.
Destroys coloring matter of blood.	1; 2		
Loss of sight			87; 601.
Loss of hearing			601.
Internal disorders			650.

The following table discloses the results of the autopsies made and reported on dogs.

TABLE II.—*Autopsies on dogs.*

Symptoms.	Unfavorable.	Favorable.	Contradictory.
No change in internal organs.			87.
Nothing abnormal		105; 399; 467; 477; 593	87 III; 457.
No change except whole interior colored red.		103	
LIVER.			
Fatty degeneration	572; 620; 639		601; 650.
Blood-poor	488 or 490		451 (2); 480 II*; 601.
Pale			451 (2).
Swollen	574		
Inflamed	649		650.
Blood-filled	574		
KIDNEYS.			
Congested	3; 639; 649		451 (2); 480* (II).
Filled with decomposed blood corpuscles.	639		451 (2).
Colored			480 (1); 601; 650.
Soft			480* II.
Blood-poor	488 or 490		601.
Blood-filled	574		
Swollen	620; 639	*692*	
Thickened			650.
Inflamed			650.
STOMACH.			
Colored	488 or 490		650.
Catarrh	488 or 490		650.
Inflamed mucous membrane.	574; 649		
Punctured mucous membrane.			601.
Contracted and filled with colored mucous.	639		
LUNGS.			
Filled with blood	574		
Punctured			601.
HEART.			
Swollen	574		
Paralyzed	574		*16* (2); 87 I; 428; 87 II.

TABLE II.—*Autopsies on dogs*—Continued.

Symptoms.	Unfavorable.	Favorable.	Contradictory.
INTESTINES.			
Colored	488 or 490		650.
Catarrh	488 or 490		
COLORED.			
Brain			650.
Fat			650.
Skin			650.
Pleura			
Heart sac			650.
Diaphragm			650.
Œsophagus	488 or 490		650.
All organs colored			480* II.
All organs swollen			480* II.
General congestion	3 (II)		
Peritonitis			70*.
Fat all disappeared	639		
Flabby muscles	639		

In this connection it may be of interest to note that out of 16 dyes producing death when administered through the mouth to dogs, 7 were on the United States market in the summer of 1907. Their Green Table numbers are as follows, the numbers in parentheses indicating the number of makers or importers, out of a possible 12: offering them: 55 (2); 86 (8); 95 (2); 428 (3); 451 (5); 601 (1); 650 (2).

The following table gives the Green Table numbers of coal-tar dyes which on administration to dogs positively did not in certain specific cases produce the particular disturbances recited, although the case of the several colors as a whole is regarded in the literature as "favorable," "unfavorable," or "contradictory," as stated.

TABLE III.—*Observations on dogs showing definite negative results in specific cases grouped under the general verdict of the literature as a whole.*

Symptoms.	Unfavorable.	Favorable.	Contradictory.
Vomiting	614	*4*; 105; 462	95; 601.
Diarrhea		*4*; 105; 462	95; 504.
Albuminuria	6; 97; 277; 434; 532; 614; 651; 659.	*4*; 92; 93; 105; 433; 462; 521; 527; 576.	14; 16; 55; 84; 86; 87; 88; 197; 398; 448; 504; 601.
Sugar in urine		*4*; 394	
Phenol in urine			287.
Loss of weight		*692*	86.

That is, when these dyes were tried on dogs the observers reported in certain specified cases, positively and definitely, the absence of any of the symptoms named.

It is stated of the following colors that they produced no bad effects in certain specified cases: *4*; 9; 65; 86; 105; 188; 197; 240; 457; 572; 593. No bad effect except colored urine: 13, 95. No bad effect except albuminuria: 88, 287. No bad effect except loss of weight: 12.5 per cent (17, 18, or 41).

It is therefore true of all of these coal-tar dyes that there are conditions under which they have been observed not to produce the bad effects as above set forth; but this tabulation must not be taken to mean that these dyes can not, under any conditions whatever, produce untoward results; the reverse is true in most cases.

EXPERIMENTS ON HUMAN BEINGS.

The Green Table numbers of those colors concerning which experimental data are available on humans are as follows: 1; 2; 3; *4;* 6; 9; (17, 18, 41); 55; 65; *85;* 86; 95; 102; 103; 105; 106; *107;* 197; 427; 428; 448; 462; 532; 602; 650.

The symptoms produced are classified in the following list:

Internally administered.

Not poisonous	*4;* 9; 55; 65; *85;* 95; 102; 103;105;106;*107;*448;462	Bad taste in mouth	86
Poor general condition	86; 650	Restlessness	86
Fever	2	Rush of blood to head	86
Loss of appetite	2	Vertigo	86; 650
Vomiting	3; 532; 650	Headache	650
Intestinal irritant	650	Delirium	650
Diarrhea	532; 650	Twitching of muscles	650
Bladder irritant	650	Yellow coloration of skin	3
Colored urine	86; 95; 650	Yellow-colored mucous membrane	3
Albuminuria	650	Food colored with it made a family sick	3
Increased micturition	650	Adults withstand	1
Irritant	532	Children and weak adults do not withstand	1
Inflammation	532	Deaths	2; 3
Dryness of throat	86	Autopsy	[1] 3

It should be noted that of the 13 dyes here classed as not poisonous to humans all but No. 102 were on the United States market in the summer of 1907, as is shown in the following table:

Number of dealers offering these nonpoisonous colors in 1907.

Green Table numbers.	Sources offering same.	Green Table numbers.	Sources offering same.	Green Table numbers.	Sources offering same.	Green Table numbers.	Sources offering same.
[1] 4	10	65	2	103	6	107	7
9	1	[1] 85	2	105	1	448	4
55	2	95	2	106	5	462	2

[1] On permitted list, Food Inspection Decision 76.

Symptoms produced by external application of certain colors (Green Table numbers).

Burning	2; 427; 428	Dermatitis	*4*
Itching	2; 6; 427; 428	Eczema	(17, 18, or 41); 197; 602
Blisters	2; 6	Inflammation	427; 428
Swelling	427; 428		

It should be noted that No. 86 has been tried on humans and has been found not to produce diarrhea or vomiting.

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[1] Hemorrhagic gastritis.

G. T. 3 has apparently killed a human at 60 mg per kilo and the autopsy disclosed hemorrhagic gastritis. G. T. 448 has been suggested as a possible remedy for Bright's disease. It should also be borne in mind that adults can stand G. T. 1 in doses of 540 to 900 mg daily for a long time, whereas children and weak adults stand that substance only poorly.

EXPERIMENTS ON SMALL ANIMALS.

Results of experimenting on rabbits with 10 coal-tar dyes whose Green Table numbers are 1, 2, 86, 89, *107*, 427, 448, 504, 517, and 563 have been tabulated as follows:

Death	1; 2; 427*; 563*	Paralysis	427*
Diarrhea	1	Cramps	427*
Colored urine	86	No harm produced	89; *107;* 448; 504; 517
Softened feces	86		

In the case of the numbers marked with an asterisk the color was administered hypodermically. In this connection reference should also be had to the paper of Penzoldt abstracted in Section VIII, page 55.

The four coal-tar dyes 55, 103, 425, and 480 have been tested on guinea pigs and no disturbance was noticed in all, but in the case of 103 occasional thirstiness was observed. No. 448 has been fed to hens without damage, and No. 2 has been recommended as an insecticide, a fungicide, and a mouse poison.

GENERAL STATEMENTS.

The following statements may be of interest before the detailed compilation is read:

1. O. Buss (*Forschungsber. über Lebensmittel, 1896, vol. 2, pp. 163–197, 237*), in a paper entitled "Contributions to the Spectrum Analysis of some Toxic and Pharmacognostically Important Coloring Matters, with Special Consideration to the Ultra-Violet," cites as poisonous the following (Green Table numbers follow in parentheses wherever connection could be satisfactorily established):

Picric Acid	(1)	Metanil Yellow	(95)
Dinitro o- and p-Cresol	(2)	Corallin	(483)
Martius Yellow	(3)	Safranin	(584)
Aurantia	(6)	Methylene Blue	(650)
Fast Yellow	(8)	Iodin Green	(459)
Orange II	(86)	Alizarin Blue S	(563)

As nonpoisonous:

Naphthol Yellow	(4)	Naphthol Green	(398)
Eosin	(512)	Malachite Green	(427, 428)
Erythrosin	(517)	Dahlia	(450, 451)
Anilin Blue	(457)		

As doubtful:

Methyl Violet	(451, 454)	Vesuvin	(197, 201)

On the following Buss is noncommittal:

Auramin	(425)	Magenta	(448)
Biebrich Scarlet	(163)	Aurin	[1](483)
Water blue	(480)	Acid Green	(435)

Naphthol Black of various brands, which seemed to be mixtures.

2. It has been pointed out that the following nine colors are harmless:

Naphthol Yellow	(4)	Wine Green	(?)
Naphthol Brown	(?)	Milling Red	(?)
Chinolin Yellow	(667)	Azo-acid Blue	(36)
Pyrotin RRO	(115)	Fastrose	(?)
Acid Green	(434, 435)		

Most of these have not been examined experimentally, but scientific studies have been made of the poisonous qualities of Azo-Blue and Naphthol Yellow. (*Zts. angew. Chemie, 1896, p. 24.*)

3. Chlopin in his monograph (see p. 75) says:

> On the basis of my personal experience I consider the testing of the action of coal-tar dyes on man not permissible, since such experiments may induce in the subjects of experiment more or less serious symptoms of poisoning, for which in some cases there are no antidotes at our disposal. To such accidents, in my opinion, only the experimenter himself may subject himself, because he knows what he is doing. Preliminary tests of dyes on dogs and other animals afford no guaranty of escape from disagreeable accidents which may take place in the testing of the dye on man. (*Page 111.*)
>
> These data and calculations convincingly prove how erroneous the current opinion is that for the coloring of food substances and beverages only exceedingly small, almost unweighable, quantities of dyes are used. (*Page 113.*)

On page 221 et seq., the following general discussion of this subject is found:

THE MANNER OF ACTION OF POISONOUS DYES ON THE ANIMAL ORGANISM.

> The mechanism and the chemistry of the action of the artificial dyes of the aromatic series on the animal organism remains to the present day, with few exceptions, exceedingly slightly and superficially studied. The same can be said also concerning the pathological and anatomical changes which are induced by these dyes.
>
> More than the others there have been studied in the toxicological respect the dyes belonging to the Nitro group; Picric Acid; Martius Yellow (Dinitro-naphthol potassium), and Saffron substitute (Dinitrocresol potassium.)
>
> According to Kobert, these dyes belong to the poisons acting on the blood. According to the same authority, Methylene Blue, which belongs to the other chemical group of Thiazins, acts similarly.
>
> In the fundamental works on the sanitary investigations for the dyes, by Cazeneuve and Lépine, by Weyl, and by Santori, we find almost no material relating to the explanation of the manner of the action of the dyes. These investigators limiting themselves to a very cursory description of the symptoms of poisoning, and reciting in most general terms the results of autopsy, not even indicating the cause of death. Such, for instance, are the reports of autopsies made by T. Weyl and some other investigators, as quoted above.

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[1] Apparently a purified form of Corallin.

We may expect that more detailed investigations in this respect will be made at the proper time by pharmacologists, since study of the mechanism and chemistry of the action of the poisonous substances on the animal organism is their province; for the hygienist it is quite sufficient merely to establish the fact that a given substance is poisonous or harmful, and he need not go any further. For this reason, in those cases in which I desired to clear up the causes of death of the animals in my experiments, and to record pathological and anatomical changes (although by the terms of the regulations governing this competition, a close study of the action of the dyes, and the ascertainment of the mechanism and chemistry of their action was not required), I called in a person more competent than myself on these questions.

Not counting the duplicates we made five autopsies all told. In all these cases death resulted from paralysis of the heart. The pathological and anatomical changes in all cases, except one, did not present anything specific, and finally reduced themselves to a feebly expressed turbid swelling of the heart and of the liver, a rush of blood to the stomach, and a congestion of the internal organs.

The exception was the autopsy of a dog, which died from Methyl Orange; this dog died with the symptoms of paralysis of a cerebro-spinal nature. This experiment was made twice, and the autopsies of both animals showed hyperæmia in the lowest part of the spinal column, on the border of the anterior and the lateral columns.

As to the symptoms of poisoning not resulting in acute death, here most frequently was observed vomiting, diarrhea, and albumen in the urine, showing disturbance of the functions of the digestive tract, and an affection of the kidneys.

A highly typical picture of poisoning is presented by the sulphid Vidal dyes. They cause rapid, almost instantaneous, deafening of the animal, whereupon the animal falls on one side in convulsions and lies, not moving its body, but convulsively and rapidly twitching its anterior limbs during several minutes. The tongue hangs out of the mouth, a strong secretion of saliva is noted, then vomiting begins, and the dog gradually begins to revive; with difficulty he arises on his front feet and sits down, not being able yet to stand on his posterior extremity, which is in a state of paresis. After a few hours the dog becomes normal. The symptoms of poisoning, just described, are exceedingly similar to the supposed "apoplectic form" of poisoning by hydrogen sulphid, which had been studied on animals by K. Lehmann, and which was observed in persons who inhaled air containing a few per cent of this gas. Air containing 0.1 to 0.3 per cent of hydrogen sulphid kills cats and dogs in 10 minutes.

In our experiments in which were introduced substances containing sodium sulphid, the poisoning must have been caused by hydrogen sulphid which was liberated from the dye by the acid of the gastric juice, and which could cause poisoning also through the stomach and through the respiratory apparatus.

Fortunately Vidal dyes, owing to their repulsive odor, will scarcely find a wide application in coloring food and beverages.

SOME REFLECTIONS REGARDING FURTHER INVESTIGATIONS OF DYES FROM A SANITARY STANDPOINT.

The present investigation, as well as all the investigations of the action of dyes on animals by previous investigators, had for its object the solution of the question in what number there exists among the dyes of the aromatic series dyes which possess poisonous, or more or less pronounced harmful properties (answering essentially the sanitary toxicological question). From the practical point of view such investigations presented and do present the most important interest, inasmuch as they afford a possibility of protecting the public from the use of obviously poisonous and harmful substances, but by such investigations questions of exceedingly sanitary importance are not answered, namely:

1. Ought we to consider as quite harmless those dyes which do not induce pronounced symptoms of poisoning and which are designated herein by the term non-poisonous?

2. Is the usual answer which is given to the hygienist by the defenders of the unrestricted use of the coal-tar dyes for coloring food products and beverages, namely, that in practice the dyes are introduced into the human organism in so small quantities that their properties can be neglected, justified?

To both questions, besides the facts and considerations which I gave above in my investigations, we may reply experimentally in two ways:

(*a*) By prolonged investigations continued over a period of years of the action of very minute quantities on the animal organism, which has so far, owing to the inconveniences of such long experiments, not been done by anybody; and

(*b*) By investigation of the action of small doses of dye on some physiological functions, and first of all on the activity of the digestive organs, which is first of all disturbed upon the introduction of dyes into foods and beverages. The solution of the last question can be best promoted, in my opinion, by experiments made on dogs and by exploratory examination of the body according to the method of Prof. Pawlow. Unfortunately, experiments such as these, owing to the difficulty of the Heidenhain-Pawlow operation, are inaccessible to the majority of investigators.

As a very useful substitute of such investigations may serve observations on the action of dyes on the activity of the digestive juices outside the body of animals.

On my proposition Dr. A. E. Winogradow began in my laboratory experiments on the action of small doses of dyes of the aromatic series on the digestion *in vitro*. Dr. Winogradow so far examined 25 coal-tar dyes in this respect according to the method of Metta and convincingly proved that in insignificant doses coal-tar dyes (from one-half to 4 milligrams) entirely stopped the digestion of albumen by artificial gastric juice. It was found that the capacity to depress the digestion is possessed not only by poisonous dyes, but also by dyes which proved in my experiments on animals nonpoisonous.

It is quite possible, therefore, that an admixture of coal-tar dyes will exert an unfavorable influence on the digestion and assimilation of food prepared from products colored by them. Experimental proof of the last supposition can be given only by experiments on the influence of dyes on the metabolism of substances in animals and man.

COMPLETE DETAILED STATEMENT OF ALL COMBINED DATA.

ABBREVIATIONS OF AUTHORITIES CITED.

The data hereinafter given is brought together as nearly as possible under the Green Table numbers to which it is pertinent. It is believed that the literature has been quite thoroughly searched, and that nothing of substantial importance has escaped recording in this compilation; certainly whatever may have escaped can hardly serve to change the general conclusion to which this compilation leads.

In order to avoid repetition in the following tabulation, "Weyl" is to be understood as referring to the book entitled "The Coal Tar Colors, with Especial Reference to their Injurious Qualities, etc.," by Theodor Weyl, translated by Leffmann and published in Philadelphia, Pa., in 1892.

"Lieber" refers to the book entitled "The Use of Coal Tar Colors in Food Products," by Hugo Lieber, published in New York in 1904.

"Fraenkel" refers to the book entitled "Arzneimittel Synthese," by Dr. Sigmund Fraenkel, published in Berlin in 1906.

"Confectioners List" refers to the Official Circular from the Executive Committee of the National Confectioners' Association of the

United States entitled "Colors in Confectionery" and reprinted, in part, in the book entitled "Food Inspection and Analysis," by Albert E. Leach, published in New York in 1906, pages 630–634.

"Resolutions of Swiss Analytical Chemists" refers to these resolutions as published in Zeitschrift für Nahrungsmittel Untersuchung und Hygiene, 1891, page 293.

"Schacherl" refers to Schacherl's publication entitled "Die Zulaessigkeit Kuenstlicher Farbstoffe zum Färben von Lebensmitteln," published in Vol. III, pages 1041–1048, of the Report of the Fifth International Congress of Applied Chemistry held in Berlin June 2 to 8, 1903.

"Chlopin" refers to Chlopin's monograph published in Russian and entitled "Coal Tar Dyes. Classification, properties, and action of artificial dyes on the animal organism, etc.," published at Dorpat in 1903, or to the abstract of Chlopin's paper printed at page 169–172 of Vol. IV of the Report of the Fifth International Congress of Applied Chemistry held in Berlin, 1903.

"Canton of Tessin" refers to the publications of the Tessin regulations published in 1897 in Zeitschrift für Untersuchung der Nahrungs und Genussmittel, page 414.

Whenever possible the doses administered have been given in milligrams per kilo and grains per 100 pounds of body weight of animal. In the case of the tabulations taken from Chlopin's monograph this was not done; but in order to render such comparative data easily available factors have been placed at the head of each tabulation; for example, under G. T. 6 (1 gram = 106 mg = 74.2 grains), which means that each gram administered amounts to 106 mg per kilo or 74.2 grains per 100 pounds of body weight of animal; by multiplying the doses given by either of the factors the corresponding comparative information is obtained.

In addition to the 106 Green Table numbers that have been examined physiologically, there are reported the results of the physiological examination of 8 coal-tar colors not in the Green Tables, of which 3 are said to be nonpoisonous, 3 are called poisonous, 1 is called harmful, and the last is said to be "not quite harmless." These 8 dyes are not included in this compilation.

TABULATION BY GREEN TABLE NUMBERS OF PHYSIOLOGICAL AND OTHER DATA.

G. T. 1.

Trade names.—Picric acid; carbazotic acid.
Scientific name.—Symmetrical trinitrophenol.
Discovered.—1771.
Shade.—Yellow. Not offered.

FAVORABLE.

Nothing.

UNFAVORABLE.

1. Prohibited by Confectioners' List.
2. Weyl (*p. 30*): "The injurious character of picric acid has long been known."
3. "In Germany its employment for coloring food is forbidden by the imperial enactment of 1888, on account of its poisonous character." (*pp. 68–71.*)
4. "The foregoing statements show that while the acid must be considered poisonous, its injurious character is far less than has generally been assumed, nevertheless, the legal prohibition of its use as a coloring matter for food or drink is just." (*p. 71.*)
5. "Erb gave a rabbit weighing 1,700 grams, 0.06 gram of potassium picrate (24.5 grains per 100 pounds) daily for 90 days; slight loss of weight and occasional diarrhea were noted, but nothing more serious." (*p. 69.*)
6. A rabbit weighing 2,065 grams died at the end of 19 days, after having taken 2.52 grams of the substance, or 854 grains per 100 pounds body weight; number of doses not stated. (*p. 69.*)
7. Weyl's experiment on a dog, weight not given: April 21–26, 0.24 gram (3.7 grains) sodium picrate daily; April 28–May 9, 0.36 gram (5.5 grains) daily; total, 5.76 grams (8.9 grains) sodium picrate; no serious disturbance; May 13, 1.2 grams (18½ grains) sodium picrate at one dose; weakness marked, diarrhea and dyspnea next day; May 14, 0.6 gram (9½ grains) caused vomiting; evening of same day, 0.36 gram (5.6 grains) given; May 15, animal lively; 0.24 gram (3.7 grains) again given, and on evening of same day 0.72 gram (11.2 grains); May 16, marked weakness of animal, and 0.16 gram (2.5 grains) given, causing vomiting; May 17, 0.17 gram (2.6 grains) given; May 18 and 19, animal definitely recovered, and aside from strong yellow tinge of the conjunctiva and skin, no abnormal conditions manifest. Animal died May 20 after receiving 1.32 grams (20.4 grains) potassium picrate. Weyl concludes, therefore, that dogs are resistant to this substance, notwithstanding the prostration and the blood disorganization.
8. Weyl summarizes the effect on humans from therapeutic and poisoning cases to the effect that daily doses of from 0.54 to 0.90 gram (8.3 to 13.8 grains) of potassium picrate are easily borne by healthy adults for a considerable time; children and weak adults bear picric acid badly. (*p. 70.*)
9. "Picric acid * * * is poisonous * * *." (*p. 96.*)
10. Fraenkel (*p. 572*): "On the other hand, this substance is not usable for internal administration on account of its decomposing the red blood corpuscles, and of its energetic cramp production, as well as on account of its disturbance of the kidneys, and the ultimate paralysis of the respiratory centers; nevertheless, picric acid is not to be considered a violent poison * * *."
11. Schacherl (*p. 1044*): "Picric acid * * * (is), according to numerous statements in the literature, poisonous even in small doses, and (is) therefore unqualifiedly to be declared as unpermissible."
12. Lieber (*p. 16*), where it is stated to be forbidden by the German law, and is also otherwise substantially the same as Weyl above quoted.
13. Resolutions of the Society of Swiss Analytical Chemists, September, 1891: "The following are to be regarded as coloring matters harmful to health: * * * picric acid * * *."
14. Prohibited by the Belgian law of June 17, 1891.
15. Lewin (*Lehrbuch der Toxikologie, 1897, p. 232*): "Picric acid is poisonous. Rabbits can stand daily 10 milligrams of a green containing picric acid, but not 20 milligrams. Their death is accompanied by paralysis."
16. Buss lists it as poisonous.

G. T. 2.

Trade names.—Victoria Yellow; Victoria Orange; Golden Yellow; Saffron Substitute; Anilin Orange; Di-nitro-Cresol.

Scientific name.—Di-nitro-o-and-p-cresol.

Shade.—Yellow. Not offered.

Discovered.—1869.

Used for coloring butter, liqueurs, etc.

FAVORABLE.

Nothing.

UNFAVORABLE.

1. Prohibited by Confectioners' List.
2. FRAENKEL (*p. 572*): "On the other hand dinitro-cresol is much more intensely poisonous (than picric acid), which is probably caused by its greater solubility in water."
3. SCHACHERL (*p. 1044*): * * * Dinitro-cresol [is], according to numerous statements in the literature, poisonous even in small doses, and [is] therefore unqualifiedly to be declared as unpermissible."
4. Resolutions of the Society of Swiss Analytical Chemists, September, 1891: "The following are to be regarded as coloring matters harmful to health * * * Dinitro-cresol * * *."
5. Forbidden by the Canton of Tessin.
6. WEYL (*p. 31*): "I have shown the same (poisonous nature) for Dinitro-cresol (Saffron Substitute). (See *Zts. angew. Chem.*, 1888, No. 12, for confirmation of my results by Gerlach.)"
7. "The reverse is the case with the poisonous dinitro-cresol (Saffron Substitute)." (*p. 55.*)
8. WEYL describes experiments with this compound. (*pp. 71–85.*)
9. Fourteen rabbits were experimented on, of which 13 died. Amounts administered in the fatal cases per 100 pounds body weight were (*p. 74*):

Grains.	Grains.
189	175
175	168
175	175
175	168
175	168
175	175
175	

Of 12 experiments on dogs, 5 receiving the color by the mouth and 7 hypodermically, 3 cases resulted fatally; the fatal case by the mouth requiring 38½ grains per 100 pounds body weight; the 2 fatal cases hypodermically represented 11 and 20 grains per 100 pounds body weight, respectively, although 140, 38.5, 31.6, and 35 grains per 100 pounds body weight by the mouth were borne without fatal effect; and 24½, 11.9, 9.8, and 4.9 grains per 100 pounds body weight, hypodermically, were also borne without fatal effect (*p. 75*).

10. WEYL (*p. 96*): "* * * Dinitro-cresol * * * are [is] poisonous; * * *"
11. Prohibited by the Belgian law of June 17, 1891.
12. (*Zts. Nahr. Genussm., 1892, p. 353*): Recommended as an insecticide, 1500 being sufficient for all ordinary purposes. One milligram is sufficient to kill a mouse; 2 milligrams recommended for killing mice.
13. WEYL (*Handbuch der Hygiene*): For humans, the fatal dose, when administered by the stomach, appears to be 60 milligrams per kilo body weight, or 43 grains per 100 pounds.

14. "The president of the Council of Oppeln forbids on April 19, 1899, the use of Saffron Surrogate for coloring food products."
15. Lewin (*Lehrbuch der Toxikologie, 1897, p. 232*): "Saffron Surrogate * * *, which is used for coloring foodstuffs, is poisonous. It appears to attack the coloring matter of the blood, and produces, in the case of dogs, vomiting, cramps, and convulsions. * * * Feathers colored with Saffron Surrogate cause burning and itching, and finally blisters on the hands of the women working with them; the faces were also similarly affected, and this was accompanied by loss of appetite and fever."
16. Buss lists it as poisonous.

G. T. 3.

Trade names.—Martius Yellow; Naphthol Yellow; Naphthylene Yellow; Naphthylamin Yellow; Manchester Yellow; Golden Yellow; Saffron Yellow; Jaune d'Or; Jaune Naphthol.

Scientific name.—Dinitro-alpha-naphthol.

Discovered.—1864.

Shade.—Yellow. Not offered.

FAVORABLE.

Nothing.

UNFAVORABLE.

1. Prohibited by Confectioners' List.
2. Weyl (*p. 31*): "Cazeneuve and Lépine pointed out the poisonous nature of Martius Yellow * * *."
3. "This body (Chamber of Commerce at Sonneberg) recommends for the preparation of children's toys three colors, the poisonous character of which I can demonstrate. These are Martius Yellow * * *." (*p. 34.*)
4. "For instance, for preliminary researches, dogs and rabbits have value for chemical reasons. The conclusions derived from such experiments must be accepted with great deliberation, since it happens that rabbits will bear without injury doses which will seriously, nay, even fatally, act upon the dog, as I have already shown to be the case with Martius Yellow." (*p. 56.*)
5. Where two experiments by Cazeneuve and Lépine are referred to, in which diarrhea, vomiting, and albuminuria were produced by this substance. (*pp. 85–89.*)
6. Weyl's own experiments on 4 dogs showed weakness, vomiting, diarrhea, and albuminuria resulting from the use of this color; the amounts of color administered per kilogram of body weight were 73, 17.5, 17.5, and 11.3 milligrams, which amount to 51, 12, 12, and 8 grains, respectively, per 100 pounds of body weight. (*p. 87.*)
7. "Martius Yellow, therefore, belongs to the injurious colors. As a coloring matter for food and drink its use should be wholly prohibited." (*p. 89.*)
8. "* * * and Martius Yellow are poisonous; * * *." (*p. 96.*)
9. Lewin (*Lehrbuch der Toxikologie, 1897, p. 231*): "Like Saffron Surrogate, it is poisonous. In an experiment on myself, using large doses, I noticed among others the general yellow coloration of the skin. In a poisoning resulting fatally after 5 hours with Martius Yellow, vomiting, yellow coloration of the skin and mucous membranes were observed; whereas the autopsy revealed, among other things, hemorrhagic gastritis. (Jacobson, Hosp. Tid., 1893, p. 765.)"

10. "Such small amounts as are used for the coloring of pastry are said to be non-poisonous. (*Vitali boll. chim. farm.*, 1893, p. 738.)" (*p. 231.*)
11. Cazeneuve and Lépine (*Compt. rend.*, *1885*, *v. 101*, *pp. 1167–1169*) say: I. "A dog received 71 milligrams per kilogram of body weight, or 50 grains per 100 pounds daily. On the second day diarrhea and vomiting ensued; loss of appetite except for milk. Thereafter it experienced difficulties in breathing; suffered albuminuria; its urine was colored; it died on the sixth day. The autopsy disclosed considerable congestion. II. A dog weighing 22 kilos received 400 milligrams (19 milligrams per kilo or 13 grains per 100 pounds); this caused a yellow vomit; next day it received 500 milligrams (27 milligrams per kilo or 17 grains per 100 pounds), which caused violent diarrhea, fever, thirst, disinclination for all food. The animal was killed; the autopsy showed badly congested kidneys."
12. Prohibited by the Belgian law of June 17, 1891.
13. Schacherl (*p. 1044*): "* * * Martius Yellow * * * [is], according to numerous statements in the literature, poisonous even in small doses, and [is] therefore unqualifiedly to be declared as unpermissible."
14. Prohibited by law in Italy. (See *Lieber*, *p. 24*.)
15. Fraenkel (*p. 572*): "This substance also shows poisonous properties, although it is less poisonous than dinitro-cresol."
16. *Resolutions of the Society of Swiss Analytical Chemists*, *September*, *1891:* "The following are to be regarded as coloring matters harmful to health * * * Martius Yellow * * *."
17. Forbidden by the Canton of Tessin.
18. Dietrich (*Zts. Nahr. Genussm.*, *1902*, *v. 5*, *p. 364*): "A lot of groats, after eating which a family became sick, was found to be free from ordinary poisons, but had been colored with Martius Yellow."
19. Buss lists it as poisonous.

DOUBTFUL.

1. Winogradow (*Zts. Nahrs. Genussm.*, *1903*, *v. 6*, *p. 589*) says it noticeably retards digestive action; is not indifferent.

G. T. 4.

Trade names.—Sulphur Yellow; Sulphonaphthol Acid Yellow; Succinic; Solid Yellow; Saffron Yellow; Jaune Acide C.; Jaune Acide; Fast Yellow; Citronin; Anilin Yellow; Acid Yellow S.

Names under which it was offered on the United States market as a food color in 1907.—Naphthol Yellow SLOZ; Naphthol Yellow S; Naphthol Yellow; Naphthol Yellow L; Yellow F Y; Lemon Yellow.

Scientific name.—Dinitro-alpha-naphthol-beta-monosulphonic acid.

Discovered and patented.—1879.

Shade.—Yellow. Offered by 10 out of 12 sources.

FAVORABLE.

1. Permitted by Confectioners' List.
2. Cazeneuve and Lépine (*Compt. rend.*, *1885*, *v. 101*, *pp. 1167–1169*): "A dog received each day for 5 days 32 milligrams per kilogram of body weight, or 23 grains per 100 pounds; for the 10 days next succeeding it received four times that amount, that is, 133 milligrams per kilogram of body weight, or 93 grains

2. **Cazeneuve and Lépine** (*Compt. rend., 1885, v. 101, pp. 1167–1169*)—Continued. per 100 pounds; for the 10 days next succeeding it received daily twice the last amount, or 266 milligrams per kilogram of body weight, that is, 186 grains per 100 pounds. It received altogether in the 25 days 62½ grams, or 964 grains. There was no vomiting, no diarrhea, and no albumen in the urine at any time."
3. **Weyl** (*p. 31*): "* * * not poisonous to human beings and dogs: Naphthol Yellow S. * * *."

   Weyl describes his own experiments on 3 dogs, giving them, respectively, 417, 34, and 100 milligrams per kilo body weight, or per 100 pounds 292, 24, and 70 grains, respectively. Whether the color was administered by the mouth, or injected subcutaneously, all bodily functions appeared to remain normal, and it was only in the case of repeated doses of 417 milligrams per kilogram of body weight, or 292 grains per 100 pounds, that albuminuria appeared. (*pp. 89–92.*)
4. "Only the sulphonated colors Naphthol Yellow * * * are harmless and applicable to the coloring of food and drink." (*p. 96.*)
5. Permitted by the laws of Austria.
6. Permitted by the law of Italy.
7. Permitted by the law of France.
8. **Schacherl** (*p. 1044*): "* * * Naphthol Yellow S * * * possesses no poisonous properties."
9. **Fraenkel** (*p. 572*): "Naphthol Yellow S is an entirely nonpoisonous substance."
10. **Meyer** (*J. Amer. Chem. Soc. 1907, v. 29, p. 900*): One hundred milligrams per kilogram of body weight for the initial administration, and subsequent administrations increased geometrically. After the second administration intermittent diarrhea resulted, emphasized by increased amounts with no albumin or sugar in the urine; continued for 14 administrations; so that in 14 administrations 147.58 grams of color had been given; the initial dose is 70 grains per 100 pounds of body weight, and the average daily dose of the total administered is 394 grains per 100 pounds of body weight. Urine only slightly yellow colored after small doses, but red after larger doses.
11. **Lieber** (*p. 143*): A dog received 36 milligrams per kilogram of body weight, or 25.2 grains per 100 pounds once a day seven times every other day; during the whole period the dog was apparently in good condition with no bad effects from the color.
12. **Buss** lists it as nonpoisonous.
13. **Cazeneuve and Lépine** (*Compt. rend., 1885, v. 101, pp. 1167–1169*): Three chronic invalids received daily from 2 to 4 grams of the dye in cochets; except slight colic and diarrhea nothing abnormal.

DOUBTFUL.

1. **Lewin** (*Lehrbuch der Toxikologie, 1897, p. 232*): "Acid Yellow S is said to be able to produce dermatitis on frequent contact therewith."
2. **Winogradow** (*Zts. Nahr. Genussm., 1903, v. 6, p. 589*) says it noticeably retards digestive action; is not indifferent.

G. T. 5.

Trade name.—Brilliant Yellow; Naphthol Yellow S or RS.
Scientific name.—Dinitro-alpha-naphthol-alpha-monosulphonic acid.
Discovered and patented.—1884.
Shade.—Yellow. Not offered.

FAVORABLE.

1. Permitted by Confectioners' List.
2. **Weyl** (*pp. 92–94*): Describes experiments on 2 dogs, in which the initial dose was 532 milligrams by the mouth, and 17 milligrams hypodermically, per kilogram of body weight, respectively, 372 and 12 grains per 100 pounds of body weight; in both cases the urine was colored, in the second case traces of albuminuria resulted. In the first case the albuminuria was doubtful.

3. "Brilliant Yellow is not poisonous, even in large doses, when administered by the stomach * * * the albuminuria was very slight. (*p. 94.*)
4. "Only the sulphonated colors * * * Brilliant Yellow, are harmless, and applicable to the coloring of food and drink." (*p. 96.*)
5. **Schacherl** (*p. 1044*): " * * * Brilliant Yellow * * * possesses no poisonous properties."
6. **Fraenkel** (*p. 572*): "For the same reason * * * Brilliant Yellow * * * is without effect."

G. T. 6.

Trade names.—Aurantia; Nitrodiphenylamin; Imperial Yellow; Kaiser Yellow.

Scientific name.—Hexanitro-diphenylamin.

Discovered.—1873.

Shade.—Yellow. Not offered.

FAVORABLE.

Nothing.

UNFAVORABLE.

1. **Weyl** (*p. 96*): " * * * Aurantia suspicious."
2. **Schacherl** (*p. 1044*): " * * * Aurantia [is] according to numerous statements in the literature poisonous even in small doses, and [is] therefore unqualifiedly to be declared as unpermissible."
3. **Chlopin** (*p. 116*) as results of his experiments considers it injurious. The experimental data are as follows:

Experimental data by Chlopin.

[1 gram=106 mg=74.2 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1902.	*Grams.*	*Kilos.*	*cc.*	
Mar. 12	1	9.4	450	Before experiment urine and dog normal.
13	2	....	425	Vomited several times; urine orange, and no albumen.
14	3	....	400	Repeated vomiting; urine dark brown, acid; no albumen; dog eats.
15	3	....	390	No vomiting; urine almost black; acid; no albumen.
18	3	....	410	No vomiting; urine dark brown, acid; no albumen.
19	3	....	400	Vomiting; urine almost black; acid; no albumen.
20	3	....	....	No vomiting; urine almost black, acid; no albumen.
21	....	....	425	Same; urine chocolate brown, acid; no albumen; general condition normal.
22-26	....	9.3	....	Gradually color of urine becomes normal; in every other respect dog is well.
Total.	18			

4. **Fraenkel** (*p. 573*): "The coloring matter called Aurantia, which is a salt of hexanitro-diphenylamin, appears to be poisonous on account of the nitro groups, which is on the other hand denied by a few observers."
5. *Resolutions of the Swiss Analytical Chemists, September, 1891:* "The following are to be regarded as coloring matters harmful to health * * * Aurantia * * *."
6. Forbidden by the Canton of Tessin.
7. **Lewin** (*Lehrbuch der Toxikologie, 1897, p. 232*): "Aurantia has a poisonous action. After wearing gloves for 8 hours made with so-called dogskin, which were colored with Aurantia, a man suffered confluent blisters, accompanied by itching. The workmen with this material get blisters on the face and on the hands. Perspiration increases the tendency to such blisters."
8. **Buss** lists it as poisonous.

G. T. 8.

Trade names.—Acid Yellow; Fast Yellow G; Acid Yellow G; Fast Yellow; Fast Yellow extra; Jaune Acide; New Yellow L.

Names under which it was offered on the United States market as a food color in 1907.—Fast Yellow Y; Fast Yellow G; Acid Yellow G pat.; Fast Yellow 053.

Scientific name.—Amidoazobenzene-disulphonate with some sodium amidoazobenzene-monosulphonate.

Discovered.—1878.

Shade.—Yellow. Offered by 5 out of 12 sources.

FAVORABLE.

1. Permitted by Confectioners' List.
2. **Cazeneuve and Lépine** (*Bull. de l'acad. de méd., April 27, 1886, p. 643*), where it is classified among the "nontoxic" colors.
3. **Fraenkel** (*p. 575*), where it is stated to be nonpoisonous.
4. Permitted by the law of Austria.

UNFAVORABLE.

1. **Weyl** (*p. 115*): "Poisonous to human beings. (?)"
2. **Chlopin** (*p. 151*) considers that the work of others makes this a suspicious color. His own experimental data are as follows:

Experimental data by Chlopin.

G. T. 8 AND 9.

[1 gram=152 mg=106 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1901.	*Grams.*	*Kilos.*	*cc.*	
Mar. 11–13	....	....	310	Dog normal; color urine normal; acid; no albumen.
14	1	6.6	....	Do.
15	....	....	....	Do.
16	....	....	....	Urine greenish brown; reddens with H_2SO_4 and HCl; no albumen.
17	....	....	305	Color same; traces of albumen.
18	....	....	380	Color less intense; trace of albumen.
19	3	6.4	320	Do.
20	....	....	280	Urine greenish brown; albumen gone; dog is lively.
21	....	6.5	292	Everything normal.
Total.....	4			

Conclusion; "Suspicious,"

3. **Buss** (*Forschungsber. über Lebensmittel, 1896, Vol. III, p. 173*): Is regarded as poisonous.
4. **Kobert** (*Lehrbuch der Intoxicationen, 1893, p. 335*): Listed as poisonous.
5. **Lewin** (*Lehrbuch der Toxikologie, 1897, p. 231*) says "produces eczema," and cites *Deutsche Med. Wochenschr., 1891, p. 45.*

G. T. 9.

Trade names.—Fast Yellow R; Fast Yellow; Yellow W.

Name under which it was offered on the United States market as a food color in 1907.—Fast Yellow 034.

Scientific name.—Sodium salt of amidoazotoluene-disulphonic acid.

Discovered and patented.—1878.

Shade.—Yellow. Offered by 1 out of 12 sources.

FAVORABLE.

1. Permitted by Confectioners' List.
2. **Weyl** (*p. 31*): " * * * not poisonous to human beings and dogs * * * Solid Yellow."
3. **Cazeneuve and Lépine** (*Compt. rend., 1885, v. 101, pp. 1167-1169*): A. A dog received 42 milligrams per kilogram of body weight, or 29 grains per 100 pounds, for 5 days; thereupon received four times that amount for 5 days, or 168 milligrams per kilogram body weight, or 117 grains per 100 pounds; for the 10 days next succeeding it received daily twice the last dose, or 336 milligrams per kilogram of body weight, that is, 235 grains per 100 pounds; it then received in 1 day 20 times the original dose, or 840 milligrams per kilogram body weight, that is, 596 grains per 100 pounds, and during the entire period nothing abnormal was noticed. B. Three chronic invalids received from 2 to 4 grains of the dye daily; except colic without diarrhea nothing abnormal. They concluded that this dye is no more harmful than Naphthol Yellow S (G. T. 4).
4. **Cazeneuve and Lépine** (*Bull. de l'acad. de méd., 1886, p. 643*): Tolerated by man, well or sick.

UNFAVORABLE.

1. **Weyl** (*p. 115*): "Poisonous to human beings. (?)"
2. **Chlopin** (*p. 151*): Where he considers that the work of others makes this a suspicious color. For his experimental data thereon see table under G. T. 8; Chlopin's chemical description of the dye used applies to both G. T. 8 and 9.
3. **Kobert** (*Lehrbuch der Intoxicationen, 1893, p. 336*): Listed as poisonous.

G. T. 11.

Trade names.—Sudan I; Carminaph.

Names under which it was offered on the United States market as a food color in 1907.—Oil Orange 7078; Cerasin Orange I.

Scientific name.—Benzene-azo-betanaphthol.

Discovered.—1883.

Shade.—Orange Yellow. Offered by 2 out of 12 sources.

FAVORABLE.

1. **Weyl** (*p. 115*): "Nonpoisonous * * * Soudan I * * *."
2. "Other Azo-colors, * * * for instance Soudan I * * * are entirely nonpoisonous." (*p. 148.*)

UNFAVORABLE.

1. Prohibited by Confectioners' List.
2. **Weyl** (*p. 119*): Dog received 18 grams in 6 doses in 17 days, and remained under observation 5 days longer; 4 doses of 168 milligrams per kilogram body weight (118 grains per 100 pounds) and 2 doses of 420 milligrams per kilogram body weight (294 grains per 100 pounds) produced colored urine, phenol in urine, vomiting and distinct albuminuria beginning with the third dose. From the foregoing Weyl concludes as follows: "The color in the doses administered is not entirely harmless, since a limited albuminuria seems to be brought about."
3. **Fraenkel** (*p. 576*): "It is not wholly harmless, since this coloring matter seems to produce a slight albuminuria."

G. T. 13.

Trade names.—Ponceau 4 G B; Crocein Orange; Brilliant Orange; Orange G R X.

Names under which it was offered on the United States market as a food color in 1907.—Crocein Orange 10234; Crocein Orange; Crocein Orange G; Ponceau 4 G B.

Scientific name.—Anilin-azo-betanaphthol-monosulphonic acid.

Discovered.—1878.

Shade.—Orange Yellow. Offered by 6 out of 12 sources.

FAVORABLE.

1. Permitted by Confectioners' List.
2. **Weyl** (*p. 115*): "Nonpoisonous * * * Ponceau 4 G B * * *."
3. Experiment on dog in doses of 161 milligrams per kilogram body weight; that is, 113 grains per 100 pounds body weight, apparently produced no disturbance aside from coloring of the urine. (*p. 124.*)
4. **Weyl** (*p. 148*): "Other Azo-colors * * * for instance * * * New Coccin * * * are entirely nonpoisonous."
5. Weyl's conclusion reads as follows: "This color can be regarded as nonpoisonous."
6. **Fraenkel** (*p. 577*): "Ponceau 4 G B can be regarded as nonpoisonous."

UNFAVORABLE.

1. Excluded by law of Austria.

G. T. 14.

Trade names.—Orange G; Orange G G.

Names under which it was offered on the United States market as a food color in 1907.—Orange G G crystals; Orange G.

Scientific name.—Anilin-azo-betanaphthol-disulphonic acid G.

Discovered and patented.—1878.

Shade.—Orange Yellow. Offered by 2 out of 12 sources.

FAVORABLE.

1. Chlopin examined this color, and his experimental data are as follows:

Experimental data by Chlopin.

No. 1 (*p. 123*).

[1 gram=73 mg=51 grains.]

Date.	Dose.	Weight.	24-hours' urine.	General condition of animal and urine.
1901.	*Grams.*	*Kilos.*	*cc.*	
Apr. 4	1	13.7	525	Nothing abnormal; no albumen.
5			600	Urine light chocolate brown, acid; no albumen.
6	2		900	Urine clear, yellow, blackish sheen; no albumen.
7			550	Urine clear, dark brown; no albumen; faintly alkaline.
8			680	Do.
9			730	Nothing abnormal.
10			755	Do.
Total.	3			

No. 2 (*p. 124*).

[1 gram=119 mg=108 grains.]

1901.				
Nov. 8		8.4	315	Nothing abnormal; no albumen.
12	3			Do.
13-14			370	Urine orange in color, acid, no albumen; in other respects nothing abnormal.
15	3			Do.
16	3		250	Do.
17	3		320	Do.
18	3		210	Do.
20			225	Do.
21			295	Do.
Total.	15			

Conclusion: Nonpoisonous.

UNFAVORABLE.

1. Excluded by Austrian law.

G. T. 15.

Trade names.—Ponceau 2 G; Orange R.

Scientific name.—Sodium salt of benzene-azo-beta-naphthol-disulphonic acid R.

Shade.—Bright Red. Not offered.

FAVORABLE.

1. Permitted by Confectioners' List.

UNFAVORABLE.

1. Excluded under Austrian law.
2. Excluded under Swiss laws.

G. T. 16.

Trade names.—Butter Yellow; Oil Yellow.

Scientific name.—Dimethyl-amido-azo-benzene.

Discovered.—1875.

Shade.—Yellow. Not offered.

FAVORABLE.

1. **Weyl** (*p. 31*): "Butter Yellow produces no disturbance in rabbits."

UNFAVORABLE.

1. **Chlopin** (*p. 138*): Where as a result of his own experiments he considers it poisonous. His experimental data are as follows:

Experimental data by Chlopin.

No. 1.

[1 gram=159 mg=111 grains.]

Date.	Dose.	Weight.	General condition of animal and urine.
1902.	*Grams.*	*Kilos.*	
May 6	3	6.3	Before experiment urine normal; after a few hours vomiting; urine not collected.
7-8	....	....	No vomiting; eats poorly; more tired than usual.
9-15	....	....	Dog gradually becomes normal.
16	3	....	Vomiting; loss appetite.
17	....	....	Repeated vomiting.
18	....	....	Vomiting continues.[1]

No. 2.

[1 gram=96 mg=67 grains.]

1903.			
Feb. 5	[2] 3	10.4	Before experiment dog quite normal; acid; no albumen.
6	....	....	Vomiting during night; dog does not eat, but drinks; urine more yellow than normal; acid; no albumen; in the evening the dog walked and drank water.
7	....	....	Died during the night; thin excreta in kennel; autopsy showed paralysis of heart as causing death.

No. 3.

[1 gram=167 mg=117 grains.]

1903.			
Feb. 8	[2] 2	6	Five hours after administration vomiting and involuntary thin feces.
9	....	....	Dog does not eat; drinks much; weak; urine dark yellow; no vomiting.
10	....	....	Do.
11	....	....	Dog lies in cage; moves slowly when allowed out of cage; in the evening retching.
12	....	....	Dog is still weak, but general condition somewhat better; urine strikingly colored; acid; no albumen.
13	....	....	Began to eat; no albumen.
14-16	....	....	General condition improving and almost normal; lassitude continues more than usual; urine normal color; acid; no albumen.
17	[3] 2	....	In the evening repeated vomiting; loss of apetite.
18	2	....	No vomiting; in the evening bothersome; does not eat.
19	....	5.7	Stools normal; striking disturbance of movements; paralysis of legs; must spread hind legs to stand; when pushed not only falls, but turns on his back; sight and hearing are normal; dog died at night.

[1] This dog ran away. [2] Gruebler's make. [3] Berlin make.

G. T. 17, 18, 41.

17. *Trade names.*—Chrysoidin Y; Chrysoidin crystals.

Name under which it was offered on the United States market as a food color in 1907.—Chrysoidin Y.

Scientific name.—Anilin-azo-meta-phenylene-diamin.

Discovered.—1875.

Shade.—Orange. Offered by 2 out of 12 sources.

18. *Trade names.*—Chrysoidin R; Cerotin Orange; C extra; Gold Orange for cotton.

Name under which it was offered on the United States market as a food color in 1907.—Chrysoidin R.

Scientific name.—Anilin-azo-meta-tolylene-diamin.

Shade.—Yellow brown. Offered by 1 out of 12 sources.

41. *Trade name.*—Chrysoidin R.

Scientific name.—Hydrochlorid of toluene-azo-meta-tolylene-diamin.

Discovered.—1876.

Shade.—Orange Brown.

(NOTE.—It has not been possible accurately to differentiate in the literature as to whether Nos. 17, 18, or 41, or all three, were referred to.)

FAVORABLE.

1. WEYL (*p. 115*): "Nonpoisonous * * * Chrysoidin * * *."
2. Permitted by the law of Italy.

UNFAVORABLE.

1. Prohibited by Confectioners' List.
2. WEYL (*p. 126*): Experiment on dog, giving him 113 milligrams per kilogram body weight, or 79 grains per 100 pounds body weight, produced a slight albuminuria.

   In a second experiment a dog receiving "1 grain" ("grain" is evidently a misprint for "gram" and will be so treated), that is, 105 milligrams per kilogram body weight, or 74 grains per 100 pounds body weight, daily for one month, did not produce albuminuria, but caused a loss of body weight of about 12.5 per cent. A third dog receiving about 2 milligrams per kilogram body weight, or about 1.5 grains per 100 pounds body weight subcutaneously, suffered a loss of 20 per cent of its body weight in 23 days; dose repeated 24 days after and animal kept under observation seven days longer when animal was normal.
3. Weyl in summarizing experiments on these three dogs concludes as follows: "Chrysoidin produces, according to my investigations, a slight albuminuria, and notable reduction in body weight, but further disturbance has not been noted." (*p. 127.*)
4. FRAENKEL (*p. 577*): "The above-mentioned Chrysoidin * * * produces a slight albuminuria, and a notable decrease of body weight, and produces factory eczema."
5. LEWIN (*Lehrbuch der Toxikologie, 1897, p. 231*): "Produces eczema," and cites *Deutsche Med. Wochenschr., 1891, p. 45.*

G. T. 28.

Trade name.—Archil Substitute V.

Scientific name.—Sodium salt of para-nitrobenzene-azo-alpha-naphthylamin-para-sulphonic acid.

Discovered and patented.—1878.

Shade.—Red. Not offered.

FAVORABLE.

1. Permitted by Confectioners' List.
2. **Weyl** (*p. 115*): "Nonpoisonous * * * Archil Substitute * * *."
   Describing experiments on three dogs, as follows: A. 430 milligrams per kilogram body weight, or 301 grains per 100 pounds body weight, on each of two successive days, and double the dose on the fourth day, producing no vomiting, but a tendency to vomit, a slight albuminuria and colored urine. B. 182 milligrams per kilogram body weight, or 127 grains per 100 pounds body weight, administered daily for one month; results similar to foregoing, but no colored urine. C. 105 milligrams per kilogram body weight, or 116 grains per 100 pounds body weight administered subcutaneously, produced only slight albuminuria, and no reduction in body weight. (*p. 125.*)

G. T. 43.

Trade names.—Orange GT; Orange RN; Orange O; Orange N.

Scientific name.—Sodium salt of toluene-azo-beta-naphthol-sulphonic acid.

Discovered.—1879.

Shade.—Orange. Not offered.

FAVORABLE.

1. Permitted by Confectioners' List.

UNFAVORABLE.

1. Excluded by Austrian law.
2. Excluded by Swiss laws.

G. T. 55.

Trade names.—Ponceau R; Ponceau 2 R; Ponceau G and GR; Xylidin Red; Xylidin Scarlet.

Names under which it was offered on the United States market as a food color in 1907.—Scarlet; Orange R.

Scientific name.—Sodium salt of xylene-azo-beta-naphthol-disulphonic acid.

Shade.—Scarlet. Offered by 2 out of 12 sources.

FAVORABLE.

1. Permitted by Confectioners' List.
2. **Weyl** (*p. 31*): "According to Cazeneuve and Lépine's experiments the following are not poisonous to human beings and dogs * * * Ponceau R * * *."
3. "Ponceau R (Ponceau 2 R, Xylidin Red, Xylidin Ponceau), not poisonous to dogs neither by administration by stomach nor injection into blood." (*p. 115.*)
4. "Other Azo colors * * * Xylidin Red * * * are entirely nonpoisonous." (*p. 148.*)
5. **Fraenkel** (*p. 575*): "That the monazo coloring matters examined by Cazeneuve and Lépine, as already above stated, were nonpoisonous, can be easily explained by the constitution of these substances. These two investigators examined * * * Ponceau R * * *."

6. **Lieber** (*p. 140*): A guinea pig received 310 milligrams per kilogram body weight or 217 grains per 100 pounds body weight, once a day six times every other day; the appetite appeared to remain good, and no disturbances were noted.
7. Permitted by Austrian law.
8. Permitted by Swiss laws.
9. **Cazeneuve** and **Lépine** (*Bull. de l'acad. de méd., 1886, p. 643*): Tolerated by man well or sick.
10. **Chlopin** (*p. 150*) classes it as nonpoisonous on his own experiments. His experimental data are as follows:

Experimental data by Chlopin.

[1 gram=141 mg=99 grains.]

Date.	Dose.	Weight.	24-hours' urine.	General condition of animal and urine.
1902.	*Grams.*	*Kilos.*	*cc.*	
May 3	2	7.1	370	Dog and urine normal and no albumen.
4	....	....	351	Urine rose-colored; no albumen.
5	2	....	420	Color normal; no albumen.
6	....	....	290	Rose-colored urine; no albumen.
7	2	....	359	Normal color; no albumen.
8	....	....	360	Rose-colored; no albumen.
9	....	....	330	Color and composition normal.
Total.	6			

UNFAVORABLE.

1. Prohibited by the ordinance of the police commissioner in France. (See *Lieber, p. 30.*)
2. **Meyer** (*J. Amer. Chem. Soc. 1907, v. 29, pp. 900–901*): The dog experimented on showed signs of paralysis on the morning of the seventh day at 8 o'clock, and died at 10.40 a. m., after having received a total of 32 grams of color, of which 16 had been given on the last day. The initial dose was 70 grains per 100 pounds body weight; the total weight of color was 5,818 milligrams per kilogram body weight, or 4,073 grains per 100 pounds; the average daily dose was therefore 582 grains per 100 pounds, or 831 milligrams per kilogram body weight.

DOUBTFUL.

1. **Winogradow** (*Zts. Nahr. Genussm., 1903, v. 6, p. 589*) says it almost completely inhibits digestion.

G. T. 65.

Trade names.—Fast Red B; Bordeaux B; Bordeaux BL; Bordeaux R extra.

Names under which it was offered on the United States market as a food color in 1907.—Bordeaux B; Claret Red.

Scientific name.—Alphanaphthylamin-azo-betanaphthol-disulphonic acid.

Discovered.—1878.

Shade.—Red. Offered by 2 out of 12 sources.

FAVORABLE.

1. Permitted by Confectioners' List.
2. **Weyl** (*p. 115*): "Bordeaux Red (Fast Red B) not poisonous to human beings."
3. "Other Azo colors * * * Fast Red B * * * are entirely nonpoisonous." (*p. 148.*)
4. **Fraenkel** (*p. 575*): "That the monoazo coloring matters examined by Cazeneuve and Lépine, as already above stated, are nonpoisonous, can be easily explained by the constitution of these substances. These two investigators examined * * * Bordeaux B."
5. **Arloing and Cazeneuve** (*Archives de physiologie, 1887, pp. 356–393*): As a result of this work, which is divided into three parts—(1) Stating the effect of direct introduction of the color into the circulation; (2) intravenous injections; (3) comparing the effects of injections of color and of salt; and (4) feeding by the mouth—these investigators conclude that these coloring matters are toxic only in extremely large doses; that when given to dogs with their food that no inconvenience of any kind results; this is based upon experiments on three dogs, covering 145 days, where each dog received per kilogram of initial body weight in the first case 20,307 milligrams, or 14,213 grains per 100 pounds initial body weight; in the second case, 29,590 milligrams, or 20,713 grains per 100 pounds initial body weight; and in the third case, 28,154 milligrams, or 19,758 grains per 100 pounds initial body weight. Per day this means 98 grains per 100 pounds initial body weight in the first case; in the second case, 143 grains per 100 pounds initial body weight; and in the third case, 137 grains per 100 pounds initial body weight.
6. **Cazeneuve** (*Arch. gén. de méd., 1886, p. 753*) says it may be taken without effect by man or animals, sick or well, in large doses.
7. **Cazeneuve and Lépine** (*Bull. de l'acad. de méd., 1886, p. 643*): Tolerated by man well or sick.

G. T. 70.

Trade name.—Azarin S.

Scientific name.—Ammonium bisulphite compound of dichlorophenol-azo-beta-naphthol.

Shade.—Red. Not offered.

FAVORABLE.

1. Permitted by Confectioners' List.
2. **Weyl** (*p. 115*): "Nonpoisonous * * * Azarin S * * *."
3. Experiments on five dogs; three fed by the mouth; two treated hypodermically, of which latter one died. The first dog received 1,367 milligrams per kilogram body weight in 25 days; that is, 54.7 kilograms per day on the average, or a total of 957 grains per 100 pounds of body weight; that is, 38 grains per 100 pounds body weight per day. The second dog received a total of 1,942 milligrams per kilogram body weight in 20 days, or 97 milligrams per kilogram body weight per day, which amounts to a total of 1,359 grains per 100 pounds body weight, or 68 grains per 100 pounds body weight per day. In both cases a distinct amount of albumen was present in the urine, and the urine evolved sulphurous acid on treatment with hydrochloric acid. The third dog received hypodermically three doses in eight days, each dose being 213 milligrams per kilogram body weight; that is, 149 grains per 100 pounds body weight; no bad effects. (*p. 133.*)
3. "Administered by the stomach Azarin S is harmless." (*p. 134.*)
4. "Other Azo colors, * * * for instance Azarin S, are entirely nonpoisonous." (*p. 148.*)
5. **Fraenkel** (*p. 578*): "Azarin S administered by the stomach is entirely harmless."

UNFAVORABLE.

1. **Weyl** (*p. 134*): Dog; weight not given; received 5 cc of Azarin S paste by injection into the abdominal cavity, and survived three days. "The cause of death was considered to be peritonitis without effusion. The result of this post-mortem is of much interest. The red spots consisted, as was determined by chemical analysis, of the azo color which is the basis of the Azarin S. Consequently in the peritoneal cavity the same splitting up of the Azarin S had occurred which takes place when it is attached to textiles."
2. **Fraenkel** (*p. 578*): To the same effect.

G. T. 78.

Trade name.—Erika B.

Scientific name.—Sodium salt of methyl-benzenyl-amido-thio-xylenol-azo-alpha-naphthol-disulphonic acid.

Discovered and patented.—1889.

Shade.—Rose Pink. Not offered.

FAVORABLE.

Nothing.

UNFAVORABLE.

1. **Chlopin** (*p. 153*): Based on his own experiments considers it as not harmless. The experimental data are as follows:

Experimental data by Chlopin.

[1 gram=125 mg=87.5 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1901.	*Grams.*	*Kilos.*	*cc.*	
May 9	2	8.0	392	Dog normal; urine acid; no albumen.
10	....	....	400	Urine of rose shade; insignificant traces of albumen; acid.
11	....	....	420	Color same; no albumen.
12	2	....	360	Do.
13	....	....	390	Do.
14	....	....	293	Urine yellow, greenish shade; traces of albumen.
15	....	....	350	Color normal; no albumen.
16	2	8.2	390	Do.
17	....	....	402	Urine wine yellow, orange, acid; no albumen.
18	....	....	350	Dog quite well; urine orange; no albumen.
Total.	6			

G. T. 84.

Trade names.—Resorcin Yellow; Tropæolin O; Tropæolin R; Chrysoin; Chryseolin; Yellow T; Gold Yellow; Acme Yellow.

Names under which it was offered on the United States market as a food color in 1907.—Chrysoin REZ; Resorcin 0275.

Scientific name.—Sodium salt of para-sulphobenzene-azo-resorcinol.

Discovered.—1875.

Shade.—Reddish yellow. Offered by 2 out of 12 sources.

FAVORABLE.

1. **Chlopin** (*pp. 131–2*) examined this color physiologically, and has classified it as harmless. The experimental data are as follows:

Experimental data by Chlopin.

No. 1 (*p. 227*).

[1 gram=43 mg=30 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1901.	*Grams.*	*Kilos.*	*cc.*	
Apr. 9	....	23.00	500	Dog quite well; urine normal color, acid; no albumen.
10	[1]1.21	....	....	Do.
11	[2]0.70	....	....	Urine acid; no albumen.
12	[1]2.00	....	....	No symptoms of poisoning; eats.
13	....	....	460	Urine dark brown; no albumen.
14	....	....	520	Do.
17	....	....	580	Urine normal color; no albumen; dog is well.
Total..	[1]3.21 [2]0.70			

No. 2.

[1 gram=156 mg=109 grains.]

1901.				
Oct. 9	....	6.4	30	Dog well; urine yellow; no albumen.
10	2	....	350	Do.
11	2	....	....	Urine brown, acid; no albumen.
12	....	....	300	Urine light brown, acid; no albumen.
13	2	....	335	Do.
14	....	....	290	Do.
15	....	....	290	Do.
16	2	....	375	Do.
17	....	....	300	Do.
18	2	....	....	Do.
19	....	....	320	Urine light brown, acid, no albumen; diarrhea.
20	....	....	360	Brown yellow, acid, no albumen no diarrhea.
21	....	....	360	Urine normal; dog is well.
22-26	....	7.0	....	Do.
Total...	10			

[1] Internally. [2] Subcutaneously.

2. Permitted by the law of Italy.

UNFAVORABLE.

1. Forbidden by the Confectioners' List.

DOUBTFUL.

1. **Winogradow** (*Zts. Nahr. Genussm., 1903, v. 6, p. 589*) says it noticeably retards digestive action; not indifferent.

G. T. 85.

Trade names.—Orange I; Alphanaphthol Orange; Naphthol Orange; Tropæolin OOO; Orange B.

Names under which it was offered on the United States market as a food color in 1907.—Orange RZ; Orange 027.

Scientific name.—Sodium salt of para-sulphobenzene-azo-alpha-naphthol.

Discovered.—1876.

Shade.—Orange. Offered by 2 out of 12 sources.

FAVORABLE.

1. Permitted by Confectioners' List.
2. **Weyl** (*p. 31*): "According to Cazeneuve and Lépine's experiments, the following are not poisonous to human beings and dogs: * * * Orange * * *."
3. "Orange I (Alpha-naphthol Orange, Tropæolin OOO) not poisonous to dogs neither by administration by stomach, nor by injection into blood." (On authority of Cazeneuve and Lépine.) (*p. 115.*)
4. **Weyl** (*pp. 123, 148*) refers to this as not poisonous.
5. Permitted by the law of Italy.
6. Permitted by the law of Austria.
7. **Cazeneuve** (*Arch. gén. de méd., 1886, Vol. I, p. 753*) says it may be taken without effect by man or animals, sick or well, in large doses.
8. **Cazeneuve and Lépine** (*Bull. de l'acad. de méd., 1886, p. 643*): Tolerated by man, well or sick.

G. T. 86.

Trade names.—Orange II; Betanaphthol Orange; Tropæolin OOO No. 2; Mandarin G extra; Chrysaurein; Gold Orange; Orange extra; Atlas Orange; Orange A.

Names under which it was offered on the United States market as a food color in 1907.—Naphthol Yellow SLOZ; Orange II; Orange; Orange Y; Mandarin G extra; Orange A 1201; Orange A extra.

Scientific name.—Sodium salt of para-sulphobenzene-azo-beta-naphthol.

Discovered.—1876.

Shade.—Orange. Offered by 8 out of 12 sources.

FAVORABLE.

1. Permitted by law in Italy.
2. **Frentzel** (*Zts. Nahr. Genussm., 1901, v. 4, p. 974*) says that according to his experiments this color, in the small amounts in which it is used in food products and which can enter the human system in the course of 24 hours, can hardly, even with frequent administration, cause a harmful effect.
3. **Frentzel** (*Zts. Nahr. Genussm., 1901, v. 4, pp. 968–974*): Experimented on rabbits, giving dye with food, a total of 21 grams in 19 days, a total of 8,748 milligrams per kilogram body weight, or 6,133 grains per 100 pounds body weight, in doses of 1 gram each, daily for the first 15 days, or about 417 milligrams per kilogram of body weight, or 292 grains per 100 pounds body weight per dose for these 15 doses; the color could only be detected in the urine, and the feces became softer. A dog was given 1,020 milligrams per kilogram body weight, or 714 grains per 100 pounds body weight, and showed distinct kidney irritation, great thirst, and diarrhea; recovery required about one week; and thereafter the same animal was fed by the mouth one-twentieth of the above dose each day for 9 successive days without any untoward effect. A second dog received per kilogram of body weight 172 milligrams, or 121 grains per 100 pounds body weight, and it, like the first dog, in the first experiment, showed kidney irritation, diarrhea, and great thirst. On humans 100 milligrams, or 1½ grains, colored the urine within 15 minutes, and this color remained for 24 hours; there was no sign of vomiting or diarrhea; the bitter taste of the color was noticeable.

UNFAVORABLE.

1. Forbidden by Confectioners' List.
2. Forbidden by Swiss Analytical chemists.
3. Weyl (*p. 115*): "Poisonous Orange II. * * *."
4. "Betanaphthol Orange is, therefore, according to Experiment I, poisonous in small doses when administered by the stomach, and suffices to kill an ordinarily large strong dog." (*p. 123*)
5. A. Weyl's own experiments on two dogs, the initial dose in one case being 476 milligrams per kilogram body weight, or 333 grains per 100 pounds body weight; the animal receiving in the course of 20 days, in four doses, 1,333 milligrams per kilogram body weight, or 933 grains per 100 pounds body weight; or 335 milligrams per dose per kilogram body weight; that is, 225 grains per dose per 100 pounds body weight. The animal died, and suffered diarrhea and albuminuria, and its urine was colored red throughout the entire period. B. The second dog received hypodermically per kilogram body weight, 116 milligrams, or 61 grains per 100 pounds body weight; its urine was colored; albuminuria, diarrhea, loss of hair, abscesses, and loss of weight occurred. It required 36 days to recover from four doses administered during one week. C. A rabbit received 1,333 milligrams per kilogram body weight, or 933 grains per 100 pounds body weight, and died within 12 hours. (*p. 122*)
6. "Of the 23 Azo colors subjected to examination only two * * * Orange II produce(s) such effects when administered by the stomach that we can consider it poisonous. With dogs the lethal dose is less than 1 gram per kilo of the body weight of Orange II * * *." (*p. 147.*)
7. "Further Orange II, which is poisonous * * *."
8. "The poisonous qualities of Orange II."
9. "Further, in spite of the presence of the sulpho groups, colors may be poisonous, as is shown with Orange II." (*p. 148.*)
10. Chlopin (*Zts. Nahr. Genussm., 1902, v. 5, p. 241*): A. A dog received 349 milligrams per kilogram body weight, or 244 grains per 100 pounds body weight for the first day of experiment, which dose was repeated on the third and fourth days; nothing untoward is noted for the first three days in the condition of the dog; the urine was dark red but free from albumen; on the fifth and sixth days two-thirds of the above amount was given, and on the seventh and eighth days the original dose was given. On the fourth day the animal was frisky and had a good appetite but was vomiting; on the fifth day vomiting stopped, but diarrhea ensued, which diarrhea continued for one week; the weight remained practically constant; the urine was colored throughout from dark red to orange red and dark brown and became normal the fourth day after the last administration. B. Humans: Chlopin took 200 milligrams, or 3$\frac{1}{10}$ grains, in a gelatin capsule at 3 p. m.; at 4.30 p. m. the urine was colored a strong red orange; at 6 p. m. a dryness of throat and bad taste in mouth appeared; at 6.30 p. m. felt very badly; vertigo and unable to remain seated and continue writing; blood rushed to head; the general condition very poor; somewhat improved by moving about in open air; 7.30 felt so poorly took Glauber's salt as an antidote; 11 p. m. ill condition still continuing; urine normal yellow; midnight recovered.

    Chlopin states that he would not repeat this experiment on himself, or on any other human, and he concludes therefore that this color must be regarded as harmful.
11. Chlopin (*p. 133*) classes it as "harmful." The experimental data are as follows:

Experimental data by Chlopin.

[1 gram=116 mg=81 grains.]

Date.	Dose.	Weight.	24-hours' urine.	General condition of animal and urine.
1901.	*Grams.*	*Kilos.*	cc.	
May 27	3	8.6	350	Dog is quite well; urine normal.
28			340	Urine brown red, acid; no albumen.
29	3		350	Do.
30	3		350	Diarrhea and vomiting at night, no albumen; urine orange red, acid, no albumen.
31	2		325	Diarrhea continues; no albumen; no vomiting.
June 1	2		330	Diarrhea continues; dog lively, eats with relish; no albumen.
2	3		350	Urine brown orange, acid, no albumen; diarrhea continues.
3	3	8.2	315	Urine clear; no albumen; diarrhea continues.
4			375	Urine dark brown; no albumen; diarrhea decreases.
5				Diarrhea still less; no albumen.
6		8.59		Diarrhea very slight; urine slightly orange, no albumen.
8			350	Urine normal in color and composition; diarrhea stopped.
Total..	19			

12. Buss lists it as poisonous.

DOUBTFUL.

1. Winogradow (*Zts. Nahr. Genussm., 1903, v. 6, p. 589*) says it almost completely inhibits digestion.

G. T. 87.

Trade names.—Orange III; Helianthin; Tropæolin D; Methyl Orange; Dimethylanilin Orange.

Scientific name.—Sodium salt of para-sulphobenzene-azo-dimethylanilin.

Discovered.—1876.

Shade.—Orange Yellow. Not offered.

FAVORABLE.

1. Permitted by the law of Italy.

UNFAVORABLE.

1. Chlopin (*pp. 145, 146*) on his own experiments classes it as poisonous. The experimental data are as follows:

Experimental data by Chlopin.

No. 1.

[1 gram=163 mg=114 grains.]

Date.	Dose.	Weight.	24-hours' urine.	General condition of animal and urine.
1901.	*Grams.*	*Kilos.*	cc.	
Aug. 29		6.14	310	Before experiment dog quite normal; color urine usual, acid, no albumen.
30	2		250	Do.
31			330	Color of urine dark yellow; no albumen.
Sept. 1	2		305	Urine brown; reddish with sulphuric acid; no albumen.
2			305	Do.
3				Do.
4	2			Do.
5	2			Do.
6	2		Little.	Urine dark brown; no albumen; vomiting.
7			Little.	Paresis of hind legs; dog does not eat, but drinks with avidity.
8-9			Little.	Complete ataxia, which became general on the 9th; dog can not move in straight line, and walks in circles, and drops his head on things and falls; after falling arises with difficulty and stands with widely spread feet, continuously lifting now one then the other; extremities shake; on 11th day sat with difficulty; placed on his feet, maintained equilibrium with difficulty; involuntarily lifts one paw and moves it about in the air; animal can still swallow; eyes respond to light; does not eat; on the 12th the hind legs completely paralyzed; on the 14th vomiting and paralysis of the front legs; lies quietly without moving; is killed. Autopsy shows no change in internal organs except hyperæmia in the lumbar region of the "spinal column (?) bordering between anterior and lateral columns," causing death by paralysis of the heart.
Total..	10			

Experimental data by Chlopin—Continued.

No. 2.

[1 gram = 149 mg = 104.3 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1901.	*Grams.*	*Kilos.*	*cc.*	
Dec. 3	3	6.7	....	A few hours after giving color dog in tremors; does not eat.
4	....	....	Little.	Urine black; acid; no albumen; paralysis of the extremities.
5	....	....	Little.	Dog lies in cage in full paralysis; died before dinner; no albumen in urine; cause of death, paralysis of heart.

2. Meyer (*J. Amer. Chem. Soc., 1907, v. 29, p. 900*): Dog receiving 113 milligrams per kilogram body weight; that is, 79 grains per 100 pounds body weight. Result, diarrhea, which continued throughout 17 days, although only 3 doses of the same size were given in 6 days; thereafter the dose was increased geometrically; the diarrhea continued; the coloring matter was discharged in the urine and the feces; there was no abnormal condition revealed by the autopsy.

DOUBTFUL.

1. Winogradow (*Zts. Nahr. Genussm., 1903, v. 6, p. 589*) says it noticeably retards digestive action; is not indifferent.

G. T. 88.

Trade names.—Diphenylamin Orange; Orange IV; Tropæolin OO; Orange M; Fast Yellow; Orange G S; New Yellow; Orange N; Acid Yellow D.

Scientific name.—Sodium salt of para-sulphobenzene-azo-diphenylamin.

Discovered.—1876.

Shade.—Orange Yellow. Not offered.

FAVORABLE.

1. Weyl (*p. 115*): "Nonpoisonous * * * Diphenylamin Orange * * *
2. * * * Diphenylamin Orange is * * * nonpoisonous." (*p. 132.*)
3. "For instance, the poisonous Metanil Yellow corresponds to the nonpoisonous Diphenylamin Orange." (*p. 148.*)
4. The experiments on which Weyl based the conclusions above may be summarized as follows: A. A dog received 183 milligrams per kilogram body weight, or 128 grains per 100 pounds body weight. The urine was rendered black, and contained traces of phenol and abundant albumen; this condition lasted for 72 hours, at the end of which 110 milligrams per kilogram body weight, or 77 grains per 100 pounds body weight, were administered; urine became colorless and albumen diminished. Five days afterwards a total of 10 grams, or 366 milligrams per kilogram body weight, or 256 grains per 100 pounds body weight, were administered; the urine became abundant, was strongly black, alkaline, contained albumen. The loss of weight was 1 kilogram, or about 3½ per cent. B. A dog received 308 milligrams per kilogram, or 216 grains per 100 pounds body weight, as the initial dose; albuminuria did not result until after repeated dosing same as initial dose, and administered for 2 weeks.
5. Weyl's conclusions are as follows: "According to the above investigations, Diphenylamin Orange causes albuminuria, but further disturbances did not appear during the several weeks' observations on the animals used,"

6. **Chlopin** (*p. 148*) examined this color and classes it as nonpoisonous. The experimental data are as follows:

Experimental data by Chlopin.

[1 gram=145 mg=100 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1901.	*Grams.*	*Kilos.*	*cc.*	
Oct. 27	3	7	300	Dog quite normal; urine color normal; no albumen.
28				Urine dark brown; acid; no albumen.
29	3			Do.
30	3			Do.
31	3			Do.
Nov. 1	3			Urine yellow, with sulphuric acid red; no albumen.
2	3		340	Orange with orange sheen; no albumen.
3	3	6.5		Do.
4			275	Urine brown yellow; acid; no albumen.
5	3		300	Do.
6		6.7		Do.
Total.	24			

7. **Fraenkel** (*pp. 577, 578*) to the same effect as Weyl, as above.
8. Permitted by the law of Italy.

UNFAVORABLE.

1. Prohibited by Confectioners' List.

G. T. 89.

Trade names.—Brilliant Yellow S; Yellow WR; Curcumin.

Names under which it was offered on the United States market as a food color in 1907.—Brilliant Yellow S.

Scientific name.—Sodium salt of para-sulphobenzene-azo-diphenylamin-suphonic acid.

Shade.—Yellow. Offered by 1 out of 12 sources.

FAVORABLE.

1. **Liebær** (*p. 136*): A very young rabbit received six doses on alternate days, each dose amounting to 320 milligrams per kilogram of body weight, or 224 grains per 100 pounds of body weight. No untoward symptoms are recorded; the body weight increased almost 10 per cent in 11 days.

UNFAVORABLE.

Nothing.

G. T. 92.

Trade names.—Azo Acid Yellow; Azoflavin; Azo Yellow; Indian Yellow.

Scientific name.—Mixture of nitrated diphenylamin yellow with nitro-diphenylamin.

Discovered.—1880.

Shade.—Yellow. Not offered.

FAVORABLE.

1. **Chlopin** (*p. 128*) examined this color and classifies it as nonpoisonous. The experimental data are as follows:

Experimental data by Chlopin.

No. 1.

[1 gram=125 mg=87.5 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1901.	*Grams.*	*Kilos.*	*cc.*	
Apr. 6	2	8	370	Dog normal; urine acid; no albumen.
8			Little.	Urine reddish orange; acid; no albumen.
10	3			Do.
11			600	Urine red; no albumen.
12			430	Urine almost normal color; acid; no albumen.
Total.	5			

Conclusion: Nonpoisonous.

No. 2 (*p. 129*).

[1 gram=143 mg=100 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1901.	*Grams.*	*Kilos.*	*cc.*	
Apr. 12	2	7	430	Dog normal; urine acid; no albumen.
14	3		610	Do.
15			600	Urine dark yellow; acid; no albumen.
16			400	Urine almost normal color; acid; no albumen.
Total.	5			

Conclusion: Showed no harmful effects.

2. Permitted by law in Italy.

UNFAVORABLE.

Nothing.

DOUBTFUL.

1. **Winogradow** (*Zts. Nahr. Genussm., 1903, v. 6, p. 589*) says it almost completely inhibits digestion; noticeably retards digestive action; is not indifferent.

G. T. 93.

Trade name.—Azo-fuchsin G.

Scientific name.—Sodium salt of para-sulphobenzene-azo-dioxy-naphthalene-sulphonic acid.

Discovered.—1889.

Shade.—Reddish brown. Not offered.

FAVORABLE.

1. **Chlopin** (*pp. 126, 127*) examined this color and classifies it as nonpoisonous. The experimental data are as follows:

Experimental data by Chlopin.

No. 1.

[1 gram=117 mg=82 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1901	*Grams.*	*Kilos.*	*cc.*	
May 3	2	8.5	300	Dog well and normal; urine acid; no albumen.
4			363	Urine chocolate brown; acid; no albumen.
5	2		390	Do.
6			380	Urine dark brown, chocolate, in thin layer greenish sheen; acid; no albumen.
7	2		285	Urine slightly greenish; acid; no albumen.
8			420	Dark chocolate brown; acid; no albumen.
9		8.7	292	Urine normal; dog is well.
Total.	6			

No. 2.

[1 gram=125 mg=87.5 grains.]

1901.				
Oct. 9		8.0	390	Dog quite normal; urine acid; no albumen.
10	2		310	Do.
11	2			Urine slightly more yellow; no albumen.
12			380	Urine dark brown; acid; no albumen.
13	2		325	Do.
14				Do.
15	2		396	Do.
16	2			Do.
17			397	Do.
18	2		325	Do.
19			380	Urine almost black; acid; no albumen.
20			246	Urine almost black; acid; no albumen; urine of lighter color.
21		8.4	420	Do.
Total.	12			

UNFAVORABLE.

Nothing.

DOUBTFUL.

1. WINOGRADOW (*Zts. Nahr. Genussm., 1903, v. 6, p. 589*) says it almost completely inhibits digestion.

G. T. 94.

Trade names.—Tartrazin; Hydrazin Yellow.

Names under which it was offered on the United States market as a food color in 1907.—Flavazein Red Shade Z; Tartrazin; Acid Yellow AT.

Scientific name.—Sodium salt of benzene-azo-pyrazalone-carboxy-disulphonic acid.

Discovered.—1884.

Shade.—Yellow. Offered by 6 out of 12 sources.

DOUBTFUL.

1. LIEBER (*p. 134*): Dog, 2 months old, received, per kilo, body weight, 37 milligrams, or 26 grains per 100 pounds body weight, six times, on alternate days. The animal suffered from mild diarrhea at the start, which continued with greater or less activity than at the start throughout the test.

2. **Meyer** (*J. Amer. Chem. Soc., 1907, v. 29, p. 897*): Dog received 100 milligrams per kilogram body weight, or 70 grains per 100 pounds, increased in geometric proportion for 6 consecutive days, at the end of which time diarrhea set in. On that day 2,000 milligrams per kilogram body weight, or 1,400 grains per 100 pounds body weight, were administered; this was about two-thirds as much as the animal had received in all the 5 days preceding; the feces were colored after the first administration, and the urine was also colored; albuminuria doubtful.
3. **Fraenkel** (*pp. 210 and 216*): Tartrazin, according to the Green Tables, is a derivative of isopyrazolon; "* * * only those substances which are derivatives of pyrazolon are antipyretics, the isopyrazolon derivatives are, however, poisonous." Tartrazin is also closely related to the antipyrin class of compounds, which class is known to possess an irritant action and also a depressing action on the circulation. Tartrazin also contains benzol groups, which are said to increase the physiological activity of pyrazolon derivatives.

G. T. 95.

Trade names.—Metanil Yellow; Orange MN; Tropæolin G.

Names under which it was offered on the United States market as a food color in 1907.—Victoria Yellow conc. Z; Yellow MXX conc.

Scientific name.—Sodium salt of meta-sulphobenzene-azo diphenylamin.

Discovered.—1879.

Shade.—Orange. Offered by 2 out of 12 sources.

FAVORABLE.

1. **Frentzel** (*Zts. Nahr. Genussm., 1901, v. 4, p. 974*): A. A rabbit received 379 milligrams per kilogram body weight, or 265 grains per 100 pounds body weight; no color administered for 2 days, and then administered at intermittent periods, so as to receive 6 doses in 19 days. Total weight administered per kilogram body weight 2,085 milligrams, or 1,460 grains per 100 pounds, which is equivalent to 77 grains per day per 100 pounds; the color could always be detected in the urine, and the feces became softer. B. A dog received 581 milligrams per kilogram body weight at one dose; that is, 407 grains per 100 pounds body weight. There was no vomiting and no diarrhea; the dye persisted in the urine for 72 hours. C. The same dog, after recovering from the foregoing, received daily one-tenth the above dose for 9 days; the dyestuff was found in the urine and the feces and movements were normal; dissection showed nothing abnormal. D. A human swallowed 100 milligrams, or 1.5 grains; no untoward symptoms are recorded; the urine remained colored for 24 hours. From the foregoing Frentzel concludes that this coloring matter is absolutely harmless.
2. **Chlopin** (*Zts. Nahr. Genussm., 1902, v. 5, p. 241*): A. A dog received 305 milligrams per kilogram body weight; that is, 104 grains per 100 pounds body weight; no color administered for four days; the same dose was given on alternate days six times, and the dose was increased 50 per cent, and that dose administered twice, and the original dose was given on the next alternate day; altogether the animal received per kilogram body weight, 3,355 milligrams, or 1,144 grains per 100 pounds body weight, in a period of 22 days, or an average of 153 milligrams per kilogram body weight per day, or 52 grains per day per 100 pounds body weight. At the end of the twelfth day albuminuria set in, and it required three weeks after ceasing the administration of the color for the albuminuria

2. **Chlopin** (*Zts. Nahr. Genussm., 1902, v. 5, p. 241*)—Continued.
to disappear; the urine was colored throughout the period of dosing, and it was not until 10 days after the last color had been administered that the urine regained normal color; otherwise the animal was well throughout. B. Humans: Two hundred milligrams, or $3\frac{1}{12}$ grains taken at 3 p. m., October 30, 1901, colored the urine so highly yellow that it could be dyed with. Apart from the bitter taste of the product no untoward symptoms are recorded. C. Chlopin's conclusion (*Zts. Nahr. Genussm., 1902, v. 5, p. 244*): "Metanil Yellow is not poisonous to dogs in doses of from 2 to 3 grams per day, nor to humans in doses of 0.2 grams per day, and may therefore perhaps be regarded as nonpoisonous from a practical point of view."

UNFAVORABLE.

1. **Weyl** (*p. 115*): "Poisonous * * * Metanil Yellow."
2. Says the product smelled strongly of diphenylamin. (*p. 130.*)
3. A. "Metanil Yellow must be considered poisonous when administered by the stomach from the indications of Experiments 1 and 2. The lethal dose, which is determined by Experiment 2, is 0.53 grams per kilo body weight." This lethal dose is 371 grains per 100 pounds body weight. B. A dog received 862 milligrams per kilogram body weight, or 603 grains per 100 pounds body weight. This caused vomiting; the same dose was repeated 24 hours afterwards, the animal again vomiting. The animal died within 96 hours from the first administration. C. A dog received 89 milligrams per kilogram body weight, or 62 grains per 100 pounds body weight; the urine became colored about 96 hours after administration; 5 days after the first administration the animal was given 10 times the original dose; vomiting set in within one hour; in 24 hours the urine was deeper colored; a week later after the dose last preceding, one-half of that dose was given, and the animal died within 24 hours; the animal had lost during this period approximately one-quarter its original weight. (*p. 132.*)
4. "* * * Metanil Yellow * * * produce[s] such effects when administered by the stomach that we can consider them [it] poisonous." (*p. 147.*)
5. "The poisonous qualities of * * * Metanil Yellow; the poisonous Metanil Yellow." (*p. 148.*)
6. **Fraenkel** (*p. 578*): "A dog weighing 11 kilograms was killed by 20 grams of this coloring matter within four days, whereas the isomeric Diphenylamin Orange is nonpoisonous, and it must first be considered if the poisonous nature of this substance can be explained by the easy liberation of diphenylamin from it, since this coloring matter, in and of itself, has a strong odor of diphenylamin."
7. **Chlopin** (*p. 141*): Based on his own experiments considers it "Not quite harmless." The experimental data are as follows:

Experimental data by Chlopin.

[1 gram = 153 mg = 107 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1901.	*Grams.*	*Kilos.*	*cc.*	
May 18		6.55	390	Quite normal.
19	2		350	Urine unusual color; acid; no albumen.
20			308	Do.
21			300	Urine orange; acid; no albumen.
22	2		370	Do.
23				Do.
24	2		380	Do.
25			332	Urine dark brown, no albumen.
26	2		320	Do.
27			365	Do.

Experimental data by Chlopin—Continued.

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1901.	*Grams.*	*Kilos.*	*cc.*	
May 28	2		330	Urine dark brown, no albumen.
29			350	Do.
30	2		350	Do.
31			355	Do.
June 1	2		400	Urine brown; traces of albumen.
2			340	Do.
3			330	Do.
4	3		400	Do.
5			305	Do.
6	3		390	Do.
7			330	Do.
8	2		335	Do.
9–29		6.5		Color of urine normal June 18, on 29th albumen disappears. Dog is well.
Total..	22			

8. **Chlopin** (*p. 141*): On authority of others not stated, classes this color as harmful or poisonous.
9. Prohibited by the laws of Italy.
10. Prohibited by Confectioners' List.
11. Prohibited by the Resolutions of the Society of Swiss Analytical Chemists, September, 1891.
12. Buss lists it as poisonous.

DOUBTFUL.

1. **Winogradow** (*Zts. Nahr. Genussm., 1903, v. 6, p. 589*) says that it noticeably retards digestion.

G. T. 97.

Trade names.—Orange T; Mandarin G R; Orange R; Kermesin Orange.

Name under which it was offered on the United States market as a food color in 1907.—Orange 2 R.

Scientific name.—Sodium salt of sulpho-ortho-toluene-azo-beta-naphthol.

Shade.—Orange. Offered by 1 out of 12 sources.

FAVORABLE.

Nothing.

UNFAVORABLE.

1. **Chlopin** (*p. 125*): Based on his own experiments considers this color as "harmful." The experimental data are as follows:

No. 1.

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1901.	*Grams.*	*Kilos.*	*cc.*	
Apr. 4	1		310	Nothing abnormal.
5			280	Urine reddish brown; traces of albumen; acid.
6	3		420	Do.
7			570	Urine dark brown; acid; traces of albumen.
8			370	Color of urine weaker; no albumen.
9			250	Almost normal-colored urine; no albumen; dog is well and lively.
10			330	Nothing abnormal.
Total..	4			

No. 2.

[1 gram = 217 mg = 152 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1902.	*Grams.*	*Kilos.*	*cc.*	
Jan. 25	2	4.6	200	Soon after giving color heavy vomiting and diarrhea; dog does not eat or drink the rest of the day; urine acid; no albumen.
26	....	....	....	Vomiting and diarrhea continued; dog is tired and run down, and began to eat; urine dark brown and no albumen.
27	....	....	....	Urine same; dog livelier.
28	2	4.3	180	Diarrhea and vomiting stopped; urine of brown color; acid; no albumen.
29	2	....	200	Urine orange; no albumen; acid.
Total..	6			

G. T. 102.

Trade names.—Fast Red; Roccellin; Cerasin; Rubidin; Fast Red A; Rauracienne; Orcellin No. 4.

Scientific name.—Sodium salt of para-sulphonaphthalene-azo-beta-naphthol.

Shade.—Brownish red. Not offered.

Discovered and patented.—1877.

FAVORABLE.

1. Permitted by the law of Italy.
2. Permitted by the law of Austria.
3. **Cazeneuve** (*Arch. gen. de méd., 1886, p. 753*) says it may be taken without effect by man or animals, sick or well, in large doses.
4. **Cazeneuve and Lépine** (*Bull. de l'acad. de méd. 1886, p. 643*): Tolerated by man well or sick.

G. T. 103.

Trade names.—Azorubin S; Carmoisin; Azo Acid Rubin; Fast Red C; Azorubin A.

Names under which it was offered on the United States market as a food color in 1907.—Claret Red RZ; Cardinal 3 B; Azorubin; Carmoisin B.

Scientific name.—Sodium salt of para-sulphonaphthalene-azo-alpha-naphthol-para-sulphonic acid.

Discovered and patented.—1883.

Shade.—Red. Offered by 6 out of 12 sources.

FAVORABLE.

1. Permitted by Confectioners' List.
2. **Cazeneuve and Lépine**: Not poisonous to human beings.
3. **Meyer** (*J. Amer. Chem. Soc. 1907, v. 29, p. 898*): One hundred milligrams per kilogram body weight, or 70 grains per 100 pounds body weight administered, increased geometrically; diarrhea was marked only after administration of exceptionally large doses (the seventh day); the stools were deep violet and the urine was carmine, becoming dark; the autopsy developed nothing abnormal; the whole interior was of a red color.

4. **Lieber** (*p. 138*): A guinea pig received once a day six times every other day 241 milligrams per kilogram body weight, or 169 grains per 100 pounds body weight; appetite remained good throughout, and aside from an occasional thirstiness noted no untoward observations were recorded.

G. T. 105.

Trade names.—Fast Red E; Fast Red.

Names under which it was offered on the United States market as a food color in 1907.—Claret Red RZ.

Scientific name.—Sodium salt of para-sulphonaphthalene-azo-beta-naphthol-monosulphonic acid.

Discovered and patented.—1878.

Shade.—Red. Offered by 1 out of 12 sources.

FAVORABLE.

1. Permitted by Confectioners' List.
2. **Meyer** (*J. Amer. Chem. Soc. 1907, v. 29, p. 898*): Initial dose 100 milligrams per kilogram body weight or 70 grains per 100 pounds, and given three times increased geometrically once before diarrhea was observed; there was no albumen in the urine; urine was colored deep red; feces colored red at the start, at the end of the experiment chocolate brown; autopsy showed all parts to be substantially normal.
3. **Cazeneuve and Lépine** (*Compt. rend., 1885, v. 101, pp. 823–827*): A. Dog: Weight 21.5 kilo, received as follows:

Days.	Grams.	Milligrams per kilo.	Grains per 100 pounds.
32	0.500	23.2	16.2
20	2.150	100.0	70.0
8	4.300	200.0	140.0
10	5.000	232.0	162.0
5	10.000	462.0	324.0

Nothing abnormal except occasional greenish urine; no vomiting; no diarrhea. B. Man: 1. One of the experimenters took 1 gram, dissolved in wine, daily for 15 days; no effect. 2. A man aged 25, afflicted with albuminuria, received the following:

Days.	Grams.
3	0.5
2	1.0
1	2.0

Caused colic without diarrhea; amount of urine or albumen not affected. 3. Three men afflicted with Bright's disease received each daily for 8 days 1 gram; no effect on the albumen. 4. A 30-year-old man chronic invalid took 4 grams one day, 6 grams the next day; no effect was observed.

4. **Arloing and Cazeneuve** (*Archives de physiologie, 1887, pp. 356–393*): As the result of this work, which is divided into four parts: (1) Stating the effect of direct introduction of the color into the circulation; (2) intravenous injections; (3) comparing the effects of injections of color and of salt; and (4) feeding by the mouth—these investigators conclude that these coloring matters are toxic only in extremely large doses; that when given to dogs with their food no inconvenience of any kind results; this is based upon experiments upon three dogs, covering 145 days, where each dog received, per kilogram initial body weight, in the first case, 20,307 milligrams, or 14,215 grains per 100 pounds initial body weight; in the second case, 29,590 milligrams, or 20,713 grains per 100 pounds initial body weight; in the third case, 28,154 milligrams, or 19,758 grains per 100 pounds initial body weight. Per day this would mean 98 grains per 100 pounds initial body weight in the first case; in the second case the daily dose was 143 grains per 100 pounds initial body weight; and in the third case the daily dose was 137 grains per 100 pounds initial body weight.

G. T. 106.

Trade names.—New Coccin; Cochineal Red A; Brilliant Scarlet; Crocein Scarlet 4 BX.

Names under which it was offered on the United States market as a food color in 1907.—Claret Red RZ; Crocein Scarlet; New Coccin; Scarlet L; Brilliant Scarlet 4 R.

Scientific name.—Sodium salt of para-sulphonaphthalene-azo-beta-naphthol-disulphonic acid (G.).

Discovered.—1878. Offered by 5 out of 12 sources.

Shade.—Red.

FAVORABLE.

1. **Cazeneuve and Lépine.** (See *Weyl, p. 115.*)
2. **Weyl** (*p. 31*): "* * * not poisonous to human beings and dogs, * * * Purple * * *"
3. **Cazeneuve and Lépine** (*Bull. de l'acad. de méd., 1886, p. 643*): Tolerated by man, sick or well.

UNFAVORABLE.

1. Prohibited by Confectioners' List.

G. T. 107.

Trade names.—Fast Red D; Azo Acid Rubin 2 B; Fast Red E B; Bordeaux S; Amaranth.

Names under which it was offered on the United States market as a food color in 1907.—Claret Red RZ; Red; Amaranth B (Azo color similar to); Bordeaux S; Naphthol Red S; Amaranth.

Scientific name.—Sodium salt of para-sulphonaphthalene-azo-beta-naphthol-disulphonic acid (R).

Discovered and patented.—1878.

Shade.—Red. Offered by 7 out of 12 sources.

FAVORABLE.

1. **Cazeneuve and Lépine** (see *Weyl, p. 115*): Not poisonous to human beings.
3. **Lieber** (*p. 148*): A rabbit received 284 milligrams per kilogram body weight, or 199 grains per 100 pounds once a day, five times every other day, and aside from decreased appetite the second and third day of the observation period nothing untoward is noted.
4 **Weyl** (*p. 31*): "* * * not poisonous to human beings and dogs, * * * Purple * * *"
5. **Cazeneuve and Lépine** (*Bull de l'acad. de méd., 1886, p. 643*): Tolerated by man, sick or well.

G. T. 138.

Trade names.—Fast Brown G; Acid Brown.

Scientific name.—Sodium salt of bi-sulphobenzene-disazo-alpha-naphthol.

Discovered.—1882.

Shade.—Brown. Not offered.

FAVORABLE.

1. **Weyl** (*p. 134*): "The following is a summary of the results obtained with the Disazo colors submitted to test by me, viz, Fast Brown G * * *. All these proved to be nonpoisonous * * *." A. A dog weighing 9.63 kilos received 312 milligrams per kilogram body weight, or 218 grains per 100 pounds body weight; the same dose was repeated 48 hours afterwards, when diarrhea set in, and the urine was colored red; 24 hours afterwards 208 milligrams per kilogram body weight, or 146 grains per 100 pounds body weight were given; the urine was colored strongly red 24 hours thereafter. Eight days afterwards 520 milligrams per kilogram body weight, or 364 grains per 100 pounds body weight, were administered; marked diarrhea set in; 3 days later the dose given was twice the dose last given, when severe diarrhea resulted, but unchanged color appeared in the urine, and continued for 24 hours afterwards; diarrhea continued for 96 hours. Evidences of albuminuria apparently not dependable. B. A second dog, weighing 5.9 kilos, received 339 milligrams per kilogram body weight, or 237 grains per 100 pounds body weight daily throughout an entire month; diarrhea was produced after 6 days, which continued almost during the month; appetite was diminished and the loss in weight was about 20 per cent on the original. C. Weyl concludes as follows: "According to these experiments, this color in continuous, though slight, doses, or in large doses, but less frequently, produces diarrhea, anorexia, and emaciation." (*p. 136.*)

UNFAVORABLE.

1. Prohibited by Confectioners' List.
2. **Weyl** (*p. 147*): "Of the remaining colors * * * others (produce) diarrhea (Fast Brown * * *)."

G. T. 160.

Trade name.—Crocein Scarlet 3 B; Ponceau 4 RB.

Scientific name.—Sodium salt of sulphobenzene-azo-benzene-azo-beta-naphthol-monosulphonic acid.

Discovered and patented.—1881.

Shade.—Scarlet. Not offered.

FAVORABLE.

Nothing.

UNFAVORABLE.

1. Prohibited by Confectioners' List.

DOUBTFUL.

1. HOUGHTON (*J. Amer. Chem. Soc., 1907, v. 29, pp. 1351–57*): Hinders fibrin digestion at all strengths; at 1:200 hinders casein and albumen digestion.

G. T. 163.

Trade names.—Biebrich Scarlet; Ponceau B; New Red L; Ponceau 3 RB; Fast Ponceau B; Imperial Scarlet.

Scientific name.—Sodium salt of sulphobenzene-azo-sulpho-benzene-azo-beta-naphthol.

Discovered.—1878.

Shade.—Scarlet. Not offered.

FAVORABLE.

1. Permitted by law in Italy. (See *Lieber, pp. 18, 22, 23.*)

UNFAVORABLE.

1. Prohibited by Confectioners' List.

G. T. 164.

Trade name.—Crocein Scarlet O extra.

Scientific name.—Sodium salt of sulphobenzene-azo-sulphobenzene-azo-beta-naphthol-sulphonic acid.

Discovered.—1888.

Shade.—Scarlet. Not offered.

FAVORABLE.

Nothing.

UNFAVORABLE.

1. Prohibited by Confectioners' List.

G. T. 166.

Trade name.—Wool Black.

Scientific name.—Sodium salt of sulphobenzene-azo-sulphobenzene-azo-para-tolyl-beta-naphthylamin.

Discovered.—1885.

Shade.—Black. Not offered.

FAVORABLE.

1. Permitted by Confectioners' List.
2. WEYL (*p. 134*): "The following is a summary of the results obtained with the Disazo colors submitted to test by me, viz: * * * Wool Black * * *. All these proved to be nonpoisonous * * *."

3. A. A dog received 167 milligrams per kilogram body weight, or 117 grains per 100 pounds body weight each day for 3 successive days; no color was administered for the next 2 days, and the third day the dose was double the former dose. The urine was colored bluish-black and contained albumen. Two days afterward the same dose was given; urine of intense dark-blue color, and contained unaltered coloring matter, which disappeared in 48 hours. Albuminuria continued for about 15 days. (*p. 137.*) B. "Wool Black is nonpoisonous both by gastric and by subcutaneous administration." (*p. 137.*)

G. T. 169.

Trade names.—Crocein Scarlet 7 B; Ponceau 6 RB; Crocein Scarlet 8 B.

Names under which it was offered on the United States market as a food-color in 1907.—Sodium salt of sulphotoluene-azo-toluene-azo-beta-naphthol-alpha-sulphonic acid.

Discovered and patented.—1881.

Shade.—Red. Offered by 1 out of 12 sources.

FAVORABLE.

1. Permitted by Confectioners' List.

G. T. 188.

Trade names.—Naphthol Black B; Brilliant Black B.

Name under which it was offered on the United States market as a food-color in 1907.—Naphthol Black BDF.

Scientific name.—Sodium salt of disulpho-beta-naphthalene-azo-alpha-naphthalene-azo-beta-naphthol-disulphonic acid.

Discovered and patented.—1885.

Shade.—Black. Offered by 1 out of 12 sources.

FAVORABLE.

1. Permitted by Confectioners' List.
2. WEYL (*p. 138*): A. A dog received 112 milligrams per kilogram body weight, or 78 grains per 100 pounds body weight, daily for 3 successive days; distinct albuminuria, uncolored urine; blue-colored feces; thereupon the daily dose was increased to 187 milligrams per kilogram body weight, that is, 131 grains per 100 pounds. No color was administered for 6 days, and during this time the urine was colored from reddish-violet to a bluish-black red; thereupon the last dose was doubled and 24 hours afterwards that dose was doubled; rather much albumen in urine which was bluish; albuminuria continued for about a week. B. A dog received 222 milligrams per kilogram body weight, or 155 grains per 100 pounds daily throughout a whole month; it remained entirely well with good appetite.
3. "This color is harmless when administered by the stomach, but poisonous subcutaneously." (*p. 140.*)
4. SCHACHERL: Not harmful under conditions in which it is used.

UNFAVORABLE.

1 WEYL (*p. 139*): Where a dog, receiving subcutaneously 31 milligrams per kilogram body weight, or 22 grains per 100 pounds body weight, subcutaneously, died apparently wholly as a result of the color.

2. "Naphthol Black P, however, is plainly poisonous when introduced into the subcutaneous cellular tissue." (*p. 147.*)

G. T. 197.

Trade names.—Bismarck Brown; Phenylene Brown; Leather Brown; English Brown; Manchester Brown; Vesuvin; Cinnamon Brown.

Names under which it was offered on the United States market as a food-color in 1907.—Vesuvin 4B Conc. Z; Bismarck Brown; Bismarck Brown B No. 216; Bismarck Brown B.

Scientificname.—Hydrochlorid of benzene-disazo-phenylene-diamin,

Discovered and patented.—1863.

Shade.—Reddish brown. Offered by 4 out of 12 sources.

FAVORABLE.

1. Weyl (*p. 115*): "Nonpoisonous Bismarck Brown."

UNFAVORABLE.

1. Prohibited by Confectioners' List.
2. Weyl (*p. 117*): A. A dog received 33 milligrams per kilogram body weight, or 23 grains per hundred pounds body weight; in one and one-half hours, vomiting; next day same dose same result with the addition that the animal took no food and moved about but little for 48 hours; on the fourth day same dose, in two hours, vomiting; for 96 hours animal took no food; on the eighth day albumen in the urine and the animal ate; on the ninth day 83 milligrams per kilogram body weight or 58 grains per 100 pounds body weight, vomited for one-half hour after administration; for the next 5 days the animal took hardly any food; on the fourteenth day the animal improved, took food on the fifteenth day; traces of albumen in urine for 17 days longer, at end of which time animal recovered.

   B. A dog received 169 milligrams per kilogram body weight, or 118 grains per 100 pounds body weight; in 24 hours the urine was colored brown; 48 hours after the first dose that dose was repeated, and unconverted color was found in the urine; 48 hours later the same dose was repeated; the color in the urine disappeared in 24 hours; 4 days later the dose was trebled, and the animal vomited, seemed sick for 4 days thereafter; on the fifth day recovery apparently complete. On the sixth day the last dose was repeated, and the animal vomited after the administration. It took no food for 24 hours, and was normal after 48 hours. There was no albuminuria in this case.

   C. A dog received daily for an entire month 45 milligrams per kilogram body weight, or 31½ grains per 100 pounds body weight; it was in good health during the entire time, did not vomit, and ate as usual. Its gain in weight was 6½ per cent.
3. "Bismarck Brown produces, when administered to dogs by the stomach, even in doses of 350 milligrams per kilogram body weight (245 grains per 100 pounds body weight), vomiting and albuminuria. Further disturbance is not noted even in large doses; small doses, 45 milligrams per kilogram body weight (or 31½ grains per 100 pounds body weight), even when frequently administered seem to be entirely harmless. Doses of 16 milligrams are harmless even when introduced into the subcutaneous cellular tissue." (*p. 118.*)
4. "Of the remaining colors some produce vomiting (e. g., Bismarck Brown * * *)." (*p. 147.*)

5. **Lewin** (Lehrbuch der Toxikologie, *1897, p. 231*): Produces eczema, and cites *Deutsch Med. Wochenschr., 1891, p. 45.*
6. **Fraenkel** (*p. 575*): "When the Azo dyestuffs do not contain any sulpho group they are poisonous. Thus, for example, Bismarck Brown * * *. This produces no effect in small doses; on the other hand, doses of 350 milligrams per kilogram of animal (245 grains per 100 pounds) produce albuminuria and vomiting."

DOUBTFUL.

1. **Houghton** (*J. Amer. Chem. Soc., 1907, v. 29, pp. 1351–1357*): Hinders digestion of fibrin, casein and albumen, in strengths of 1:100 or 1:400.

G. T. 201.

Trade names.—Manchester Brown EE; Bismarck Brown R; Vesuvin B.

Names under which it was offered on the United States market as a food color in 1907.—Vesuvin 4 B Conc. Z; Vesuvin B.

Scientific name.—Hydrochlorid of toluene-disazo-meta-tolylene-diamin.

Discovered and patented.—1878.

Shade.—Reddish brown. Offered by 2 out of 12 sources.

UNFAVORABLE.

1. **Lewin** (*Lehrbuch der Toxicologie, 1897, p. 231*): "Produces eczema," and cites *Deutsch. Med. Wochenschr., 1891, p. 45.*

Note.—The literature is not always conclusive as between Nos. 197 and 201, and probably most, if not all, the references under No. 197 also apply to No. 201.

G. T. 240.

Trade name.—Congo Red.

Name under which it was offered on the United States market as a food color in 1907.—Congo.

Scientific name.—Sodium salt of diphenyl-disazo-binaphthionic acid.

Discovered and patented.—1884.

Shade.—Red. Offered by 1 out of 12 sources.

FAVORABLE.

1. **Weyl**: "According to Experiments 1 and 2 Congo Red is, after long-continued administration by the stomach, harmless."
2. A. A dog received 274 milligrams per kilogram body weight, or 192 grains per 100 pounds; this dose was repeated next day, when the urine became pale, was strongly alkaline and contained albumen; the next day the same dose was repeated, whereupon the urine was of a weak red color, and a little albumen present; the next day the dose was increased to 411 milligrams per kilogram body weight, or 298 grains per 100 pounds; the urine was somewhat reddish and contained a little albumen. No color was given for 6 days, at the end of which time the urine was reddish, and deposited a reddish sediment, probably Congo. At the end of that time the dose was increased to 685 milligrams per kilogram

2. A.—Continued.
body weight, or 480 grains per 100 pounds body weight; the urine was feebly alkaline and contained some albumen. The next day the dose last given was doubled; the urine was colored to such an extent that it could be dyed with. Two days later the same dose was repeated; animal took but little food, was otherwise comfortable; there was little albumen present. The loss in body weight was about 4½ per cent. (*p. 141.*)

B. A second dog received 233 milligrams per kilogram body weight, or 163 grains per 100 pounds, daily for one month, and remained entirely well.

G. T. 269.

Trade name.—Chrysamin R.

Name under which it was offered on the United States market as a food color in 1907.—Chrysamin R.

Scientific name.—Sodium salt of ditolyl-disazo-bisalicylic acid.

Discovered and patented.—1884.

Shade.—Yellow. Offered by 1 out of 12 sources.

FAVORABLE.

1. Permitted by Confectioners' List.
2. Weyl (*p. 134*): "The following is a summary of the results obtained with Disazo colors submitted to test by me, viz: Chrysamin R." "All of these proved to be nonpoisonous, * * *."
3. A. A dog received 515 milligrams per kilogram body weight, or 361 grains per 100 pounds; urine became alkaline and yellowish, and easily dyed cotton; very little albumen. Within 24 hours the same dose was repeated, diarrhea resulting and vomiting for 3 days, whereupon the animal was given one-fifth of the dose, or 103 milligrams per kilogram body weight, that is, 72 grains per 100 pounds body weight; the urine continued slightly colored, and contained a distinct amount of albumen, continuing for 2 days, when the last dose was repeated; 24 hours afterwards the dose was doubled, and repeated the next day; the day after the dose was increased 50 per cent; 3 days later the last dose was repeated; slight albuminuria set in, lasting 5 days. B. A dog received three doses of 619 milligrams each per kilogram body weight, or 433 grains per 100 pounds body weight, three times in the course of 10 days; the urine was yellowish in color and contained very little albumen. (*p. 145.*)
4. "Chrysamin is harmless when taken into the stomach." (*p. 147.*)
5. Schacherl (*p. 1045*) says Chrysamin is harmless under the conditions in which it is used.

UNFAVORABLE.

1. Weyl (*p. 147*): "Of the remaining colors some produced vomiting * * * others diarrhea (* * * Chrysamin R * * *.")

G. T. 277.

Trade names.—Benzopurpurin 4 B; Cotton Red 4 B; Sultan Red 4 B.

Scientific name.—Sodium salt of ditolyl-disazo-binaphthionic acid.

Discovered.—1884–85.

Shade.—Red. Not offered.

FAVORABLE.

Nothing.

UNFAVORABLE.

1. Chlopin (*p. 130*): On his own experiments classes it as suspicious. The experimental data are as follows:

Experimental data by Chlopin.

[1 gram=125 mg=87.5 grains.]

Date.	Dose.	Weight.	24-hour urine.	General condition of animal and urine.
1901.	*Grams.*	*Kilos.*	cc.	
Apr. 16	2	8	300	Dog is well, lively; urine normal color; acid; no albumen.
17			330	Do.
18	3		450	Vomiting several times; no albumen.
19			470	Urine yellow-orange; no albumen; no vomiting.
20			370	Do.
21	2		320	Do.
22			310	Do.
23			370	Urine normal; dog is well.
24			310	Do.
Total.	7			

DOUBTFUL.

1. Winogradow (*Zts. Nahr. Genussm. 1903, v. 6, p. 589*) says it almost completely inhibits digestion.

G. T. 287.

Trade name.—Azo Blue.

Name under which it was offered on the United States market as a food color in 1907.—Azo Blue.

Scientific name.—Sodium salt of ditolyl-disazo-bi-alpha-naphthol-para-sulphonic acid.

Discovered.—1885.

Shade.—Grayish violet. Offered by 1 out of 12 sources.

FAVORABLE.

1. Permitted by Confectioners' List.
2. Weyl (*p. 134*): The following is a summary of the results obtained with the Disazo colors submitted to test by me, viz: * * * Azo-blue, * * * ." "All these proved to be nonpoisonous."
3. "Azo-blue is harmless, both when administered by the stomach and subcutaneously." A. A dog received 237 milligrams per kilogram body weight, or 166 grains per 100 pounds; 2 days later this same dose was repeated, and the urine was a violet color strongly alkaline and contained a little albumen. The next day the dose was increased to 2.5 times; colorless urine, and little albumen; there was no phenol. The next day 4 times the original dose was administered; abundant bluish-violet urine and little albumen. No administration for 4 days, when 2.5 times the original dose were given, and that dose repeated 3 days later; during this time the urine was colorless and contained little albumen; there was a very slight increase in weight. B. A dog received 319 milligrams per kilogram body weight, or 233 grains per 100 pounds daily for one month; animal remained well with good appetite; a slight amount of albumen made its appearance in the urine. (*p. 144.*)

UNFAVORABLE.

1. **Weyl** (*p. 144*): "A slight amount of albumen made its appearance in the urine."

G. T. 394.

Trade names.—Dinitrosoresorcin; Dark Green; Russian Green; Alsace Green; Fast Green O; Chlorin; Fast Myrtle Green.

Scientific name.—Dinitroso-resorcinol (Dioximidoquinone).

Discovered and patented.—1875.

Shade.—Green. Not offered.

FAVORABLE.

1. Permitted by Confectioners' List.
2. **Weyl** (*p. 63*): "According to the above experiments Dinitroso-resorcinol is not dangerous to dogs when administered by the stomach even in large doses; while hypodermic administration proves fatal within 24 hours, in the proportion of 190 milligrams per kilogram of body weight" (that is, 132 grains per 100 pounds).
3. A. A dog received 173 milligrams per kilogram body weight, or 121 grains per 100 pounds; scanty dark-brown urine; the next day the same dose was repeated and urine continued dark brown, and contained trace of albumen as well as distinct reaction for iron; the next day the dose was repeated; animal remained lively; the day after that the dose was increased 50 per cent; no albumen. The loss in body weight was only 4 per cent. B. A dog received 198 milligrams per kilogram body weight, or 139 grains per 100 pounds; seems to have been loss of appetite, coupled with dark-brown, almost black urine; 2 days later the dose was doubled, and the urine was colored green by ferrous oxid, and contained no albumen nor sugar; 2 days later the dose was increased 50 per cent, and some albumen was then found in the urine. (*p. 62.*)

G. T. 398.

Trade name.—Naphthol Green B.

Names under which it was offered on the United States market as a food color in 1907.—Naphthol Green; Naphthol Green B.

Scientific name.—Ferrous sodium salt of nitroso-betanaphthol-beta-monosulphonic acid.

Discovered.—1883.

Shade.—Green. Offered by 2 out of 12 sources.

FAVORABLE.

1. **Weyl** (*p. 64*): A. A dog received 172 milligrams per kilogram body weight, or 120 grains per 100 pounds body-weight; the urine was greenish, and conjunctiva stained intensely green; dose was repeated 3 successive days, and the next day the dose was increased to fivefold; appetite undisturbed, and animal remained lively. B. A dog received 417 milligrams per kilogram body weight, or 292 grains per 100 pounds; there was a dirty yellowish-green color to the urine; no albumen, and not more than traces of iron. The animal was normal within a day, and two days after the same dose was repeated; no change in animal was recorded, except that on the day following the feces were normal, but colored green.

2. **Weyl** (*p. 65*): "Experiments 1 and 2, in which 2 to 5 grams of the color were introduced directly into the stomach, demonstrated its harmlessness in this method of administration."
3. **Buss** lists it as nonpoisonous.

UNFAVORABLE.

1. Prohibited by Confectioners' List.
2. **Weyl** (*p. 65*): "On the other hand, in a hypodermic administration, in two out of three cases abscesses and septic fever were induced."

G. T. 399.

Trade names.—Sun Yellow; Curcumin S; Jaune Soleil; Maize.

Scientific name.—Sodium salt of the so-called Azoxy-stilbene-disulphonic acid.

Discovered.—1883.

Shade.—Yellow. Not offered.

FAVORABLE.

1. **Meyer** (*J. Amer. Chem. Soc., 1907, v. 29, p. 897*): A dog received 100 milligrams per kilogram body weight, or 70 grains per 100 pounds, increased geometrically through the fourth day, when diarrhea set in; up to this time the animal had been given 19.27 grams, or 1,465 milligrams per kilogram body weight, equivalent to 1,026 grains per 100 pounds body weight; the average dose per day would have been 366 milligrams per kilogram body weight, or 256 grains per 100 pounds; the animal was given its fifth portion of coloring matter the same size as the fourth, thereupon color was omitted, and for the following 7 days the dosage of the third day, which amounted to 400 milligrams per kilogram body weight, or 280 grains per 100 pounds body weight, was given; the urine was colored orange throughout the entire test after the first day; the fecal matter also was of orange color; slight diarrhea on the fourth and twelfth days of the test, and vomiting on the fifth day, the cause of which does not seem to have been definitely determined; the autopsy revealed nothing abnormal.

G. T. 425.

Trade names.—Auramin; Auramin O; Pyoctanin Aureum.

Names under which it was offered on the United States market as a food color in 1907.—Auramin O; Auramin; Canary Yellow.

Scientific name.—Hydrochlorid of imido-tetramethyl-diamido-diphenylmethane.

Discovered.—1883.

Shade.—Greenish yellow. Offered by 3 out of 12 sources.

FAVORABLE.

Nothing.

UNFAVORABLE.

1. **Chlopin** (*p. 157*): On his own experiments classes it as poisonous. The experimental data are as follows:

Experimental data by Chlopin.

[1 gram=69 mg=48 grains.]

Date.	Dose.	Weight.	24-hours' urine.	General condition of animal and urine.
1901.	*Grams.*	*Kilos.*	*cc.*	
Mar. 2-4	1.0	14.4	550	Dog quite normal; acid; no albumen.
6			340	Urine strong brown yellow; traces of albumen; appetite less.
7				Thin stool.
8				Diarrhea; urine brown yellow, pales with sulphuric acid, and contains much albumen.
9				Stool normal; color of urine weaker; much albumen; eats well; lively.
10				Stool normal; urine less colored; less albumen.
11			335	Stool normal; urine less colored; traces of albumen.
12				Stool normal; urine less colored; insignificant traces of albumen.
13				Do.
14	2.0	13.2		Do.
15				Strongly brown yellow; much albumen.
16			None.	Dog depressed; lies down; eats little.
17			295	Vomits and diarrhea; urine strong yellow; little albumen; takes only milk.
19			480	Vomits and diarrhea; urine strong yellow; much albumen.
20				Feces normal color; color urine almost normal; much albumen.
21			352	Feces normal color; color urine almost normal; albumen less.
24	[1] 0.4	13.6	380	Do.

[1] Subcutaneously.

DOUBTFUL.

1. **Winogradow** (*Zts. Nahr. Genussm., 1903, v. 6, p. 589*) says it noticeably retards digestive action; is not indifferent.

G. T. 427.

Trade names.—Malachite Green; New Green; Fast Green; Benzal Green; Diamond Green B; Malachite Green B; New Victoria Green; Vert Diamant; Bitter-almond-oil Green.

Names under which it was offered on the United States market as a food color in 1907.—Green M; New Green Crystals; Green 088.

Scientific name.—Zinc double-chlorid, oxalate, ferric double-chlorid of tetramethyldi-para-amido-triphenyl-carbinol.

Discovered.—1877-78.

Shade.—Bluish green. Offered by 2 out of 12 sources.

FAVORABLE.

1. Permitted by Confectioners' List.
2. **Weyl** (*p. 24*): "According to Grandhomme * * * Malachite Green are (is) also nonpoisonous."
3. "* * * Malachite Green are (is) as is now established, almost without poisonous action." (*p. 55.*)
4. **Lewin** (*Lehrbuch der Toxikologie, 1897, p. 231*) says when free from arsenic it is harmless.
5. **Buss** lists it as nonpoisonous.

UNFAVORABLE.

1. **Penzoldt** (*Archiv. exper. path. pharm., 1890, v. 26, p. 312*): One hundred milligrams per kilogram body weight of rabbit, or 70 grains per 100 pounds, injected subcutaneously, caused after the third day motor paralysis and occasional cramps, which resulted fatally at the end of the ninth day.

2. **Lewin** (*Lehrbuch der Toxikologie, 1897, p. 231*): In the case of one workman, in contrast with others who had long been unaffected by this substance, itching, burning, inflammation, and swelling of hands and feet, and formation of blisters occurred.

G. T. 428.

Trade names.—Brilliant Green; New Victoria Green; Emerald Green; Malachite Green B; Ethyl Green; Fast Green J.

Names under which it was offered on the United States market as a food color in 1907.—Green E; Green 087; Emerald Green Crystals.

Scientific name.—Sulphate of zinc-double-chlorid (rarely oxalate) of tetraethyl-diamido-triphenyl-carbinol.

Discovered.—1879–80.

Shade.—Yellowish green. Offered by 3 out of 12 sources.

FAVORABLE.

1. **Lewin** (*Lehrbuch der Toxikologie, 1897, p. 231*) says when free from arsenic it is harmless.
2. Buss lists it as nonpoisonous.

UNFAVORABLE.

1. **Chlopin** (*pp. 171–2*): Classifies it as "very poisonous" on his own experiments (see p. 181). The experimental data are as follows:

Experimental data by Chlopin.

No. 1.

[1 gram=125 mg=87.5 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1901.	*Grams.*	*Kilos.*	*cc.*	
Apr. 24		8	410	Before experiment dog is well; urine normal.
25	2			Soon after giving dye vomiting and diarrhea; in the evening only drank water; vomiting kept up.
26			395	No vomiting; urine greenish; acid; insignificant traces of albumen.
27				Do.
28	2		400	No vomiting; urine greenish; acid; no albumen.
29				Do.
30			395	Do.
May 1				Urine normal; dog quite well.
2				Do.
3			395	Do.
Total....	4			

No. 2.

(1 gram=111 mg=78 grains.)

1902.				
Jan. 21	3	9	400	Before experiment dog quite normal; urine normal; after giving dye very violent vomiting several times.
23–24				Urine greenish in color; acid; very much albumen; no vomiting.
25	2	7.2		Do.
26				Died during the night.
Total....	5			

Experimental data by Chlopin—Continued.

No. 3.

[1 gram=119 mg=83 grains.]

Date.	Dose.	Weight	24 hours' urine.	General condition of animal and urine.
1902.	*Grams.*	*Kilos.*	*cc.*	
Jan. 28	3	8.4	370	Before experiment dog and urine normal; soon after giving dye vomiting began, lasting over an hour; dog stands with difficulty.
29	2	....	....	During night dog improved somewhat, began to eat; drinks much; soon after giving dye vomiting.
30	....	....	....	During night vomiting and diarrhea; during night 30th, 31st, in bad condition; does not take food.
31	....	....	....	During night 31st second dog found dead in cage. Cause of death, "paralysis of the heart."
Total....	5			

2. Lewin (*Lehrbuch der Toxikologie, 1897, p. 231*): In the case of one workman, in contrast with others who had long been unaffected by this substance, itching, burning, inflammation and swelling of hands and feet, and formation of blisters occurred.

G. T. 433.

Trade name.—Guinea Green.

Name under which it was offered on the United States market as a food color in 1907.—Guinea Green B.

Scientific name.—Sodium salt of diethyldibenzyl-diamido-triphenyl-carbinol-disulphonic acid.

Discovered.—1883.

Shade.—Green. Offered by 1 out of 12 sources.

FAVORABLE.

1. Chlopin (*p. 174*): On his own experiments classified it as "nonpoisonous." The experimental data are as follows:

Experimental data by Chlopin.

[1 gram=200 mg=140 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1901.	*Grams.*	*Kilos.*	*cc.*	
May 18	2	5	250	Dog and urine normal before experiment.
19	....	....	235	Urine slightly greenish, no albumen; vomited at night.
20	....	....	280	No vomiting.
21	....	....	290	Urine slightly greenish; no albumen.
22	....	....	270	Urine normal in color and composition.
23	....	....	270	Do.
24	2	....	260	Do.
25	....	....	240	Do.
26	2	....	290	Do.
27	....	....	246	Do.
28	2	....	....	Do.
29	....	....	243	Do.
30	2	....	233	Do.
31	....	....	230	Do.
Total.	10			

G. T. 434.

Trade names.—Light Green SF bluish; Acid Green.

Scientific name.—Sodium salt of dimethyldibenzyl-diamido-triphenyl-carbinol-trisulphonic acid.

Discovered.—1879.

Shade.—Green. Offered by 1 out of 12 sources.

FAVORABLE.

Nothing.

UNFAVORABLE.

1. **Chlopin** (*pp. 176–7*): Examined this color, and on his own experiments classes it as "nonpoisonous, but not entirely indifferent." The experimental data are as follows:

Experimental data by Chlopin.

No. 1.

[1 gram=133 mg=93 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1902.	*Grams.*	*Kilos.*	*cc.*	
Apr. 24	2	7.5	380	Dog and urine normal.
25				Urine green; acid; no albumen.
26			347	Urine green; traces of albumen.
27			297	Do.
28	2			Do.
29				Urine green; no albumen.
30			360	Urine less green; no albumen.
31				Do.
May 1–3				Do.
			370	Do.
Total.	4			

No. 2.

[1 gram=110 mg=77 grains.]

1902.				
Aug. 5	3	9.1	300	Dog and urine normal.
6			310	Urine greenish, acid, no albumen.
7	3			Do.
8	3		320	Do.
9	3		350	Do.
10	3			Do.
11			400	Do.
12		9	350	Do.
Total.	15			

DOUBTFUL.

1. **Winogradow** (*Zts. Nahr. Genussm. 1903, v. 6, p. 589*) says it noticeably retards digestive action; is not indifferent.

G. T. 435.

Trade names.—Light Green SF yellowish; Acid Green; Acid Green extra conc.

Names under which it was offered on the United States market as a food color in 1907.—Acid Green conc. V N; Light Green SF yellow shade; Acid Green conc. 780; Pistachio.

Scientific name.—Sodium salt of diethyldibenzyl-diamido-triphenyl-carbinol-trisulphonic acid.

Discovered.—1879.

Shade.—Green. Offered by 4 out of 12 sources.

FAVORABLE.

1. **Lieber** (*p. 144*): The animal was a fully developed male guinea pig, and received per kilogram body weight 240 milligrams, or 168 grains per 100 pounds, five times in all, every other day. There was apparently nothing irregular or abnormal observed during the whole test of nine days.

G. T. 448.

Trade names.—Magenta; Fuchsin; Rosein; Anilin Red.

Obsolete names.—Rubin; Solferino; Fuchsiacin; Rubianite; Azalein; Erythrobenzin; Harmalin.

Names under which it was offered on the United States market as a food color in 1907.—Magenta powder A; Fuchsin Crystals; Magenta F A B S Red 101.

Scientific name.—Mixture of hydrochlorid or acetate of pararosanilin (triamidotriphenylcarbinol) and rosanilin (triamidodiphenyltolylcarbinol).

Shade.—Bluish-red. Offered by 4 out of 12 sources.

FAVORABLE.

1. Permitted by Confectioners' List.
2. **Weyl** (*p. 22*): "The colors examined * * * Fuchsin were (was) found to be nonpoisonous;" "Similarly a hen which had eaten for three weeks oats covered with fuchsin was in good health." (*p. 24.*)
3. "According to Grandhomme rabbits bear without injury fuchsin free from arsenic * * *." (*p. 31.*)
4. "Fuchsin * * * (is) as is now established, almost without poisonous action." (*p. 55.*)
5. **Fraenkel** (*p. 574*), quoting Penzoldt, says that it is entirely nonpoisonous, and completely prevents putrefaction.
6. Permitted by the law of Austria.
7. **Lewin** (*Lehrbuch der Toxikologie, 1897, p. 230*), says when free from arsenic it is harmless.
8. **Clouet and Bergeron** (*J. pharm. chim., 1871, v. 25, p. 296*): One of them took personally 500 milligrams, that is, 7.7 grains in 16 days; there was no digestive disturbance of any kind, and the urine, which was examined daily, contained no albumen. They cite a case of Bright's disease, in which the amount of albumen decreased when fuchsin was administered, and they conclude that fuchsin may be good for sufferers from Bright's disease.

UNFAVORABLE.

1. Forbidden by the law of France. (See *Lieber, p. 31.*)
2. **Chlopin** (*p. 178*): Examined this color, and on his own experiments classes it as "Suspicious because of vomiting and traces of albumen." The experimental data are as follows:

Experimental data by Chlopin.

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1901.	*Grams.*	*Kilos.*	*cc.*	
Apr. 18	3	....	270	Dog before experiment well and urine normal; vomited several times after receiving dye.
19	....	....	280	Urine quite red; acid; no albumen; general condition normal.
20	....	....	....	Do.
21	2	....	320	Urine less colored; acid; no albumen; vomited once.
22	....	....	....	Urine darkish; acid; traces albumen.
23	....	....	....	Color normal; no albumen.
24	....	....	....	Do.
Total.	5			

NOTE.—This sample may have contained some phosphin, G. T. 532, see page 133.

DOUBTFUL.

1. WINOGRADOW (*Zts. Nahr. Genussm. 1903, v. 6, p. 589*) says it almost completely inhibits digestion.

G. T. 450.

Trade names.—Hofmann Violet; Dahlia; Red Violet 5R extra; Violet R; Iodin Violet; Primula; Violet 5 R; Violet R R.

Scientific name.—Mixture of the hydrochlorids or acetates of the monodi- or trimethyl- (or ethyl-) rosanilins and pararosanilins.

Discovered.—1863.

Shade.—Violet. Not offered.

FAVORABLE.

1. WEYL (*p. 24*): "* * * Anilin Violet (Dahlia) * * * (is) also nonpoisonous."
2. BUSS lists it as nonpoisonous.

UNFAVORABLE.

1. FRAENKEL (*p. 574*) quotes Penzoldt, and says it completely arrests development, and causes muscular paralysis.

G. T. 451.

Trade names.—Methyl Violet B; Direct Violet; Dahlia; Paris Violet; Violet de Methylanilin; Pyoctanin.

Names under which it was offered on the United States market as a food color in 1907.—Methyl Violet; Methyl Violet B; Methyl Violet BB extra; Methyl Violet 3 B D.

Scientific name.—Hydrochlorid of penta- and hexamethyl- pararosanilin.

Discovered.—1861.

Shade.—Violet. Offered by 5 out of 12 sources.

FAVORABLE.

1. Permitted by Confectioners' List.
2. Permitted by the Austrian law.

3. **Weyl** (*p. 24*): "* * * Anilin Violet (Dahlia) * * * [is] also nonpoisonous."
4. "* * * Methyl Violet [is] as is now established, without poisonous action." (*p. 55*).
5. **Fraenkel** (*p. 573*): "Methyl Violet * * * is relatively nonpoisonous."
6. **Buss** lists it as nonpoisonous.

UNFAVORABLE.

1. **Graefe and Braunschweig** (*Fortschr. Medizin, 1890, v. 8, p. 405*): "It seems to be proven that damage will actually result even in the case of most cautious use, which we are sure we exercised."
2. **Santori** (*Moleschott's Untersuchungen, 1895, v. 15, p. 52*): I. A dog weighing 7,600 grams received 5.6 grams dye in 12 days; this amounts to 61 milligrams per kilo per day, or 43 grains per 100 pounds per day. Continued vomiting beginning with 0.1 gram dye; progressive emaciation and general falling away; catarrh of eyes and nose; distinct dislike for food and great desire to sleep; temperature below normal, urine unchanged. Loss of weight 1,600 grams, or 21 per cent. The animal died on the thirteenth day. The autopsy showed a pale and blood-poor liver; the kidneys were in a typically congested condition and contained accumulations of blood corpuscles. II. A second dog weighing 6,000 grams received 3.8 grams dye in 14 days, which amounts to 71.4 milligrams per kilo per day, or 50 grains per 100 pounds per day. The animal died on the fourteenth day. There was daily vomiting and rapid emaciation; final weight loss was 1,100 grams, or 18.3 per cent; temperature normal; bloody urine beginning the eighth day. The autopsy showed a blood-poor liver, soft and swollen epithelia; kidneys the same as in the case of the preceding dog.

G. T. 457.

Trade names.—Anilin Blue, spirit-soluble; Spirit Blue; Fine Blue; Bleu Lumière; Opal Blue; Gentian Blue 6B; Hessian Blue; Bleu-de-Nuit.

Scientific name.—Hydrochlorid sulphate or acetate of triphenylrosanilin and triphenylpararosanilin.

Discovered.—1860–1862.

Shade.—Greenish blue; not offered.

FAVORABLE.

1. Permitted by Confectioners' List.
2. **Santori** (*Moleschott's Untersuchungen, 1895, v. 15, p. 48*) says it is harmless. A dog weighing 4,500 grams received 17 grams dye in 30 days, which amounts to 126 milligrams per kilo per day or 88 grains per 100 pounds per day. Weight remained the same, general condition good, urine and temperature unchanged; killed by chloroform; autopsy showed everything normal.
3. **Liebber** (*p. 14*), where it is stated to be permitted by the Austrian law (*pp. 22–23*), where it is stated to be permitted by the Italian law (*p. 31*), where it is stated to be permitted by the French law in candies, pastilles, sweetmeats, sauces, fruits, and certain liqueurs ordinarily not colored.
4. **Weyl** (*p. 22*), quoting Sonnenkalb (*p. 24*), quoting Grandhomme.
5. **Fraenkel** (*p. 580*) states that it is effective in only 5 per cent of malaria cases.
6. Permitted by the law of Austria.
7. **Buss** lists it as nonpoisonous.

UNFAVORABLE.

1. **Weyl** (*p. 23*), quoting Friedrich, where poisoning was produced in a young man engaged in packing this dye.

G. T. 459.

Trade names.—Iodin Green; Pomona Green; Night Green; Vert Lumière.

Scientific name.—Zinc-double-chlorid of heptamethyl-rosanilin-chlorid.

Discovered and patented.—1866.

Shade.—Green. Not offered.

FAVORABLE.

Nothing.

UNFAVORABLE.

1. **Chlopin** (*p. 175*) on his own experiments classes it as "suspicious." (See p. 181). The experimental data are as follows:

Experimental data by Chlopin.

[1 gram=167 mg=117 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1901.	*Grams.*	*Kilos.*	cc.	
May 9	6	6	420	Before experiment dog and urine normal.
10			470	Do.
11			441	Urine slightly greenish; no albumen.
12	2		420	Do.
13			390	Urine has greenish opalescence; traces of albumen.
14			400	Do.
15			442	Urine has greenish opalescence; no albumen.
16	2		370	Do.
17				Urine has greenish opalescence; traces of albumen.
18			400	Normal color; no albumen.
Total..	6			

2. **Buss** lists it as poisonous.

DOUBTFUL.

1. **Winogradow** (*Zts. Nahr. Genussm., 1903, v. 6, p. 589*) says it noticeably retards digestive action; is not indifferent.

G. T. 462.

Trade names.—Acid Magenta; Acid Fuchsin; Acid Rubin; Fuchsin S; Acid Rosein; Rubin S.

Names under which it was offered on the United States market as a food color in 1907.—Acid Magenta Powdered; Acid Magenta.

Scientific name.—Mixture of the sodium or ammonium salts of the trisulphonic acids of rosanilin and pararosanilin.

Discovered.—1877.

Shade.—Red. Offered by 2 out of 12 sources.

FAVORABLE.

1. Permitted by Confectioners' List.
2. Cazeneuve (*Arch. gén. méd., 1886, p. 753*) says it may be taken without effect by man and animals, sick or well, in large doses.
3. Weyl (*p. 55*), where he says that it is established of this color that it is almost without poisonous action.
2. Lieber (*p. 14*), where it is stated to be permitted by the law of Austria (*p. 31*), where it is stated to be permitted by the law of France for confectionery, cordials, and the like.
5. Permitted by the law of Austria.
6. Permitted by the law of Italy.
7. Cazeneuve and Lépine (*Compt. rend., 1885, v. 101, p. 1011*): A. Dog: 15 kilos weight, received—

Days.	Grams.	Milligrams per kilo.	Grains per 100 pounds.
15	1	67	47
5	2	134	94
5	5	335	235
5	10	670	470

No diarrhea; no vomiting; no albuminuria; urine colored only occasionally, but did contain the leuco compound of the dye. B. Man: 1. Afflicted with Bright's disease; took two grams daily for one week; no effect. 2. Afflicted with renal cirrhosis; four grams daily for several days; no effect. 3. A well man took four grams daily for several days; no effect.

8. Cazeneuve and Lépine (*Bull. de l'acad. de méd. 1886, p. 643*): Tolerated by man, sick or well.

G. T. 467.

Trade name.—Acid Violet 6B.

Scientific name.—Sodium salt of dimethyl diethyl dibenzyl triamido triphenyl carbinol disulphonic acid.

Discovered and patented.—1889.

Shade.—Violet. Not offered.

FAVORABLE.

1. Santori (*Moleschott's Untersuchungen, 1895, v. 15, p. 54*): A dog weighing 4,250 grams received 12.5 grams dye in 28 days, which amounts to 105 milligrams per kilo per day, or 74 grains per 100 pounds per day. The dog vomited the color only two or three times and was otherwise normal. Appetite, temperature, and urine all remained normal. Conclusion: Nonpoisonous; autopsy also showed everything normal.

UNFAVORABLE.

Nothing.

G. T. 477.

Trade names.—Alkali Blue; Nicholson Blue; Fast Blue.

Scientific name.—Mixture of sodium salts of triphenyl rosanilin monosulphonic acid and of triphenyl para rosanilin-monosulphonic acid.

Discovered and patented.—1862.

Shade.—Blue. Not offered.

FAVORABLE.

1. SANTORI (*Moleschott's Untersuchungen, 1895, v. 15, p. 45*): A dog weighing 4,500 grams received 25 grams dye in 30 days, which amounts to 185 milligrams per kilo per day, or 129.5 grains per 100 pounds per day. The urine remained of normal color, but the stool was a deep blue black. Throughout the whole time the animal was in perfect health. Killed with chloroform; autopsy showed everything normal.

UNFAVORABLE.

Nothing.

G. T. 478.

Trade names.—Bavarian Blue DSF; Methyl Blue, water-soluble; Navy Blue B; Methyl Blue for silk MLB.

Scientific name.—Sodium salt of triphenyl pararosanilin di- and tri-sulpho acid.

Discovered.—1862; not patented.

Shade.—Blue. Not offered.

FAVORABLE.

Nothing.

UNFAVORABLE.

1. CHLOPIN (*p. 170*) examined this color, and on his own experiments classed it as "nonpoisonous but not quite indifferent."

Experimental data by Chlopin.

[1 gram = 115 mg = 81 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1901.	*Grams.*	*Kilos.*	*cc.*	
May 18	2	8.7	450	Dog and urine quite normal.
19			365	Urine slightly greenish; no albumen; acid.
23			380	Do.
24	2		370	Do.
25				Do.
26	2			Do.
27			370	Do.
28	2		320	Do.
29				Do.
30	2			Urine slightly greenish; traces of albumen; acid.
31			525	Do.
June 1				Do.
2			327	Do.
3				Do.
4	3			Do.
5				Urine slightly greenish; traces of albumen; acid; diarrhea.
6	3		405	Do.
7				Urine slightly greenish; traces of albumen; acid.
8	3		395	Do.
9			353	Do.
10	3		370	Do.
11–15			340	Color normal; traces of albumen.
16			[1] 400	Do.
17–29		8.5	425	Do.
Total.	22			

[1] Daily.

G. T. 479.

Trade names.—Methyl Blue O; Brilliant Cotton Blue greenish; XL Soluble Blue; Diphenylamin Blue; Bavarian Blue DBF; Soluble Blue 8B and 10B; Helvetia Blue.

Scientific name.—Sodium salt of triphenyl-pararosanilin trisulphonic acid.

Discovered and patented.—1862.

Shade.—Blue. Not offered.

FAVORABLE.

Nothing.

UNFAVORABLE.

1. **Chlopin** (*p. 168*) examined this color and on his own experiments classed it as "suspicious" or "nonsuspicious" dependent upon the make of goods.

Experimental data by Chlopin.

No. 1.

[1 gram=75 mg=52 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1901.	*Grams.*	*Kilos.*	*cc.*	
May 14	2	13.4	460	Dog normal; no albumen.
15				Color chocolate-brown; insignificant traces of albumen.
16	2			Color same; no albumen.
17			410	Urine normal; no albumen; dog is well.
Total..	4			

No. 2.

[1 gram=152 mg.=106 grains.]

1901.				
Nov. 12	3	6.6	300	Dog quite well; urine normal color; acid; no albumen.
13				Do.
15	3			Do.
16	3		360	Do.
17	3		305	Urine scarcely perceptible greenish sheen; acid; no albumen.
19	3		330	Urine greenish color; no albumen.
20				Urine darker; no albumen.
21				Urine color normal; no albumen.
22		6.8		Color almost normal.
Total..	15			

This preparation from Moscow; not suspicious.

No. 3 (Merck's Preparation).

[1 gram=147 mg=103 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1903.				
Feb. 18	3	6.9	400	Before experiment dog quite normal; urine acid; no albumen; normal yellow color; 2 hours after giving dye vomiting.
19	3			Vomiting stopped; diarrhea; urine chocolate-brown; reaction acid; considerable albumen; appetite not decreased.
20	3		330	During night diarrhea; no vomiting; urine brown; acid; traces of albumen; without acid urine becomes blue.
21	3			No diarrhea; no vomiting; urine brown; traces of albumen.
22	3	6.8		Urine brown, with blue sheen; traces of albumen.
23				Urine blue chocolate-brown; otherwise normal. Urine acquired usual color 8 days later.
Total..	15			

Conclusion: "Suspicious."

G. T. 480.

Trade names.—Soluble Blue; China Blue; Cotton Blue; Bleu Marine; Water Blue; Water Blue 6 B extra; London Blue extra.

Names under which it was offered on the United States market as a food color in 1907.—Pure Soluble Blue.

Scientific name.—Sodium, ammonium or calcium salt of the trisulphonic acid (with some disulphonic acid) of triphenyl-rosanilin and triphenyl-pararosanilin.

Discovered and patented.—1862.

Shade.—Blue. Offered by 1 out of 12 sources.

FAVORABLE.

1. Permitted by law of Austria.
2. LIEBER (*p. 147*): A guinea pig received 306 milligrams per kilogram body weight, or 214 grains per 100 pounds, once a day seven times every other day; the weight remained substantially constant, a slight gain of less than ¼ per cent being noted. "Nothing irregular or disturbing whatsoever was observed during the whole period."
3. SANTORI (*Moleschott's Untersuchungen, 1895, v. 15, p. 45*): A dog weighing 4,500 grams received 30 grams dye in 30 days which amounts to 223 milligrams per kilo per day or 156 grains per 100 pounds per day. Animal remained well throughout; no loss of weight or appetite; urine normal color, stool deep blue; killed with chloroform; autopsy showed a pea-green coloring of the cortex of the kidneys.

UNFAVORABLE.

1. FRAENKEL (*p. 574*), quoting Penzoldt, says that it completely arrests germ development, and causes changes internally.
2. SANTORI (*Moleschott's Untersuchungen, 1895, v. 15, p. 46*): Regards this as injurious hypodermically, but not through mouth. A dog weighing 4,000 grams received 3.5 grams of dye hypodermically in 16 days, at the end of which time he died; this amounts to 55 milligrams per kilo per day or 38.5 grains per 100 pounds per day. The autopsy showed the liver to be free from blood; kidneys soft and congested; all organs swollen and colored.

G. T. 483.

Trade names.—Aurin; Rosolic Acid; Yellow Corallin.

Scientific name.—Mixture of aurin (trioxytriphenyl-carbinol) oxidized aurin, methylaurin, and pseudo-rosolic acid (corallin phthalin).

Discovered.—1834.

Shade.—Yellowish brown. Not offered.

FAVORABLE.

1. LEWIN (*Lehrbuch der Toxikologie, 1897, p. 231*): Rosolic Acid is positively nonpoisonous.

UNFAVORABLE.

1. **Chlopin** (*p. 167*) examined this color, and on his own experiments classes it as "strongly poisonous." The experimental data are as follows:

[1 gram=137 mg=96 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1902.	*Grams.*	*Kilos.*	*cc.*	
Mar. 12	1	7.3	330	Before experiment dog quite well; urine normal color; acid; no albumen.
13	2		375	Diarrhea; urine faint red.
14	3		300	Diarrhea; lassitude; eats little; urine red; no albumen.
15	3			Violent diarrhea and vomiting; dog stands on feet with difficulty; eats nothing.
16				Same conditions as on preceding day; dog sick for a long time after.
Total..	9			

2. Prohibited by the German law of 1887.
3. Prohibited by the Belgian law of June 17, 1891.
4. Buss lists it as poisonous.

DOUBTFUL.

1. **Lewin** (*Lehrbuch der Toxikologie, 1897, p. 231*): Rosolic Acid is positively non-poisonous. Small animals can take 1 gram and more of it. In Austria it is prohibited for use in coloring foods. Corallins, or red (Pæonin) or yellow colors, consisting of Aurin and Rosolic Acid were regarded as poisonous because in experiments on man and animals illness occurred, but are said to be poisonous only in the presence of arsenic, phenol, or anilin. These substances are prohibited in the coloring of food.

G. T. 488 or 490.

Trade name.—Victoria Blue; Victoria Blue B; Victoria Blue 4R.

Scientific names.—Hydrochlorid of phenyltetra (penta) methyl-triamido-diphenyl-alpha-naphthyl-carbinol (note: 4R is bracketed).

Discovered and patented.—1883.

Shade.—Blue. Not offered.

FAVORABLE.

Nothing.

UNFAVORABLE.

1. **Santori** (*Moleschott's Untersuchungen, 1895, v. 15, p. 47*): A dog weighing 5,250 grams received 10.5 grams dye in 22 days, which amounts to 45 milligrams per kilo per day or 32 grains per 100 pounds per day. After receiving 1.5 grams the animal suffered copious continuous salivation, anemia, and emaciation and occasional vomiting. The urine's color did not change; the feces became blue; temperature slightly above and below normal; although highly emaciated (loss in weight was 1,750 grams or 33⅓ per cent) the animal retained its appetite; animal died on the morning of the 23d day. The autopsy showed œsophagus, stomach, and intestines colored deep blue and filled with a greenish scum, extended and strong catarrh of the stomach and intestines; kidneys and liver contained very little blood. Conclusion: Poisonous.

(**Note**: It is uncertain which of these two dyes Santori used.)

G. T. 502.

Trade name.—Rhodamin G and G extra.
Scientific name.—Chiefly Triethylrhodamin.
Discovered and patented.—1891.
Shade.—Bluish. Offered by 2 out of 12 sources.

FAVORABLE.

Nothing.

UNFAVORABLE.

1. Chlopin (*p. 184*) examined this color, and on his own experiments classes it as "suspicious." The experimental data are as follows:

Experimental data by Chlopin.

[1 gram=167 mg=117 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1903.	*Grams.*	*Kilos.*	*cc.*	
Feb. 18	3	6	310	Dog normal; urine acid; no albumen; after 2 hours thin stool; remainder of day lively; good appetite.
19	3	....	....	Urine fuchsin color, acid, no albumen; stool and appetite normal.
20	3	....	....	Goes to stool without results; otherwise as above.
21	3	....	270	Do.
22	3	....	320	Do.
23	....	....	....	Goes to stool without results; urine normal after 8 days.
Total..	15			

G. T. 504.

Trade names.—Rhodamin B; Rhodamin O; Safranilin.

Names under which it was offered on the United States market as a food color in 1907.—Rhodamin B extra; Rhodamin; Rhodamin B.

Scientific name.—Hydrochlorid of diethylmeta-amido-phenol-phthalein.

Discovered and patented.—1887.

Shade.—Bluish red. Offered by 5 out of 12 sources.

FAVORABLE.

1. Lieber (*p. 141*): A young female rabbit received 339 milligram body weight, or 237 grains per 100 pounds, five times on alternate days. "During the whole period the animal seemed to be perfectly at ease, was lively, displayed good appetite, and gained steadily * * * ." The gain in weight was, roughly, 7 per cent.

UNFAVORABLE.

1. Chlopin (*pp. 182, 183*) examined this color, and on his own experiments classifies it as "not poisonous, but not entirely indifferent; suspicious." The experimental data are as follows:

Experimental data by Chlopin.

[1 gram = 109 mg = 76 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1904.	*Grams.*	*Kilos.*	*cc.*	
Feb. 18	3	9.2	420	Dog normal; urine acid, no albumen; strong yellow color; 2 hours after giving color thin stool.
19	3	....	400	Urine colored wine red, strong fluorescence, disappears on boiling, and reappears on cooling; no albumen.
20	3	....	400	Thin stool; no change in other respects.
21	3	....	....	No diarrhea; no change in other respects.
22	3	....	420	General condition good; urine red and acid; no albumen.
Total..	15			

Dog recovers.

G. T. 512.

Trade names.—Eosin; Eosin A; Eosin B; Eosin A extra; Eosin Yellowish; Eosin G G F; Water-soluble Eosin; Eosin 3 J and 4 J extra.

Names under which it was offered on the United States market as a food color in 1907.—Erythrosin I N; Eosin J; Eosin Y.

Scientific name.—Alkali salts of Tetra-bromo-fluorescein.

Discovered.—1874.

Shade.—Yellowish red. Offered by 3 out of 12 sources.

FAVORABLE.

1. Permitted by Confectioners' List.
2. Weyl (*p. 31*): "According to Grandhomme, rabbits bear without injury * * * Eosin * * *."
3. Permitted by the law of Austria.
4. Buss lists it as nonpoisonous.

UNFAVORABLE.

1. Lewin (*Lehrbuch der Toxikologie, 1897, p. 231*): "The continued use of these coloring matters, as well as of Phenolphthalein, which becomes colored in the system, I regard as harmful, and, in fact, through action as coloring matters."
2. Forbidden by the Italian law.

G. T. 516.

Trade names.—Erythrosin G; Pyrosin J; Jaune d'Orient; Dianthin G; Iodeosin G.

Name under which it was offered on the United States market as a food color in 1907.—Erythrosin yellowish shade.

Scientific name.—Sodium or potassium salt of diiodofluorescein.

Discovered.—1875.

Shade.—Yellowish red. Offered by 1 out of 12 sources.

FAVORABLE.

Nothing.

UNFAVORABLE.

1. Chlopin (*p. 181*): Examined this color, and on his own experiments reports it as "injurious because of light albuminuria, vomiting, and diarrhea." The experimental data are as follows:

Experimental data by Chlopin.

No. 1.

[1 gram = 156 mg = 109 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1901.	*Grams.*	*Kilos.*	*cc.*	
Apr. 24	2	6.4	360	Before experiment dog and urine normal.
25	....	....	353	Urine red yellow with greenish sheen; acid; no albumen.
26	....	....	368	Urine red yellow with greenish sheen; acid; trace of albumen.
27	....	....	370	Do.
28	2	....	....	Urine red yellow with greenish sheen; acid; no albumen.
29	....	....	355	Do.
30	....	....	365	Do.
May 3	....	....	365	Do.
Total.	4			

No. 2.

[1 gram = 152 mg = 106 grains.]

1901.				
Nov. 24	....	6.6	345	Dog and urine normal.
25	3	....	....	Do.
26	3	....	....	Urine fluorescent orange; no albumen.
27	3	....	....	Vomiting, diarrhea; urine orange; no albumen.
28	3	....	380	No vomiting and no diarrhea; urine fluorescent, red; no albumen.
29	3	....	340	Do.
30	....	....	....	Do.
Dec. 2-3	....	....	....	Color and composition of urine normal; dog well.
Total.	15			

DOUBTFUL.

1. Winogradow (*Zts. Nahr. Genussm. 1903, v. 6, p. 589*) says it almost completely inhibits digestion.

G. T. 517.

Trade names.—Erythrosin; Erythrosin B; Iodeosin B; Eosin J; Erythrosin D; Pyrosin B; Eosin Bluish.

Names under which it was offered on the United States market as a food color in 1907.—Erythrosin Yellow Shade; Erythrosin B; Erythrosin.

Scientific name.—Sodium or potassium salt of tetraiodofluorescein.
Discovered.—1876.
Shade.—Bluish red. Offered by 5 out of 12 sources.

FAVORABLE.

1. Permitted by Confectioners' List.
2. WEYL (*p. 31*): "According to Grandhomme, rabbits bear without injury * * * Erythrosine * * *."
3. Permitted by the laws of France.
4. Permitted by the law of Austria.
5. FRAENKEL (*p. 574*): "Rose Bengal * * * produces no noticeable disturbances."
6. Buss lists it as nonpoisonous.

UNFAVORABLE.

1. LEWIN (*Lehrbuch der Toxikologie, 1897, p. 231*): "The continued use of these coloring matters, as well as of phenolphthalein, which becomes colored in the system, I regard as harmful, and, in fact, through action as coloring matters."

G. T. 520.

Trade names.—Rose Bengal; Rose Bengal A T; Rose Bengal N; Rose Bengal G.

Names under which it was offered on the United States market as a food color in 1907.—Rose Bengal B; Phloxin B.
Scientific name.—Alkaline salt of tetraiododichlorofluorescein.
Discovered.—1875.
Shade.—Bluish red. Offered by 2 out of 12 sources.

FAVORABLE.

1. Permitted by Confectioners' List.

G. T. 521.

Trade names.—Phloxin; Phloxin TA; Eosin 10B.
Scientific name.—Sodium salt of tetrabromotetrachlorofluorescein.
Discovered.—1882.
Shade.—Red. Not offered.

FAVORABLE.

1. Permitted by Confectioners' List.
2. Permitted by the Austrian law.
3. CHLOPIN (*p. 185*) examined this color, and on his own experiments classifies it as "nonpoisonous." The experimental data are as follows:

Experimental data by Chlopin.

No. 1.

[1 gram=143 mg=100 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1901.	*Grams.*	*Kilos.*	cc.	
Apr. 10	1	7.0	380	Dog and urine quite normal.
11			370	Do.
12			370	Do.
13	3		370	Do.
14				Urine orange.
15			390	Do.
Total..	4			

No. 2.

[1 gram=116 mg=81 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1901.				
Nov. 3	3	8.6	450	Dog well; urine normal color; acid; no albumen.
4			430	Urine orange; no albumen.
5	3			Do.
6			400	Do.
7	3		440	Do.
8	3		430	Do.
9			450	Do.
10	3		400	Do.
11				Color almost normal; no albumen.
12-14				Do.
Total..	15			

G. T. 527.

Trade names.—Cœrulein S; Alizarin Green; Anthracene Green.
Scientific name.—Sodium bisulphite compound of cœrulein.
Discovered.—1879.
Shade.—Black. Not offered.

FAVORABLE.

1. Chlopin (*pp. 186–7*) examined this color, and on his own experiments reports it as "nonpoisonous, and not sufficient data to regard it as suspicious." The experimental data are as follows:

Experimental data by Chlopin.

No. 1.

[1 gram=147 mg=103 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1901.	*Grams.*	*Kilos.*	cc.	
Aug. 30	2	6.8	320	Before experiment dog quite normal.
31			330	Urine normal; acid; no albumen.
Sept. 1	3		300	Do.
2	3		330	Urine has scarcely perceptible sheen; no albumen.
3			300	Do.
4	3			Do.
5	3		300	Do.
6	3			Do.
7	3			Nothing abnormal.
9			310	Do.
10	2			Do.
11			375	Do.
12-18				Do.
Total..	22			

Experimental data by Chlopin—Continued.

No. 2.

[1 gram=167 mg=117 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1902.	*Grams.*	*Kilos.*	*cc.*	
Mar. 3	2	6	270	Before experiment dog quite well; urine normal.
4	3	....	250	Urine yellow with orange sheen; acid; no albumen.
5	3	....	265	Thin stool twice; urine dark yellow with greenish sheen; acid; no albumen.
6	2	....	....	Stool normal; urine yellow green; acid; no albumen; general condition quite well.
Total..	10			

DOUBTFUL.

1. WINOGRADOW (*Zts. Nahr. Genussm., 1903, v. 6, p. 589*) says it almost completely inhibits digestion.

G. T. 530.

Trade name.—Benzoflavin.

Scientific name.—Hydrochlorid of diamido-phenyl-dimethyl-acridin.

Discovered.—1887.

Shade.—Brownish orange yellow. Not offered.

FAVORABLE.

Nothing.

UNFAVORABLE.

1. CHLOPIN (*p. 189*) on his own experiments regards this color as suspicious. The experimental data are as follows:

Experimental data by Chlopin.

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1901.	*Grams.*	*Kilos.*	*cc.*	
May 3	2	....	365	Before experiment dog well and urine normal.
4	....	....	391	Dog ate poorly; traces of albumen.
5	2	....	....	Do.
6	....	....	330	Urine greenish sheen; acid.
7	2	....	375	Color same; no albumen.
8	....	....	....	Do.
9	....	....	....	Do.
Total..	6			

G. T. 532.

Trade names.—Phosphin; Xanthin; Leather Yellow; Philadelphia Yellow G.

Scientific name.—Nitrate of chrysanilin (unsym. diamido-phenyl-acridin) and homologues.

Discovered.—1862.

Shade.—Orange yellow. Not offered.

FAVORABLE.

Nothing.

UNFAVORABLE.

1. **Chlopin** (*p. 190*) reports Fuchsin containing Phosphin as suspicious.
2. On his own experiments says this color "does not belong to the poisonous colors, but is not wholly harmless." The experimental data are as follows:

Experimental data by Chlopin.

[1 gram=147 mg=103 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1901.	*Grams.*	*Kilos.*	cc.	
Apr. 15	....	6.8	390	Dog well; urine normal.
16	2	....	350	Do.
17	....	....	350	Do.
18	3	....	....	Urine yellower than normal; vomited several times after taking dye; no albumen.
19	....	....	330	Urine same; no vomiting.
21	2	....	370	Vomited twice; urine same.
22	....	....	....	No vomiting; urine same.
23	....	....	....	Color normal.
24	....	....	....	Dog well; urine normal.
Total..	7			

3. (*p. 178*): See experimental data on G. T. 448, which also applies to this color.
4. **Fraenkel** (*p. 578*): Where its physiological action is compared with quinin its action on protozoa is far greater. "The Phosphins are locally strong irritants, and producers of inflammation of medium poisonous nature so that humans can very well bear 400 milligrams, or 6.17 grains.
5. **Lewin** (*Lehrbuch der Toxikologie, 1897, p. 232*): "Phosphin * * * produces in humans, in doses up to 1 gram, vomiting and diarrhea."

DOUBTFUL.

1. **Winogradow** (*Zts. Nahr. Genussm., 1903, v. 6, p. 589*) says it almost completely inhibits digestion.

G. T. 563.

Trade names.—Alizarin Blue S; Anthracene Blue S; Alizarin Blue ABS.

Scientific name.—Sodium bisulphite compound of dioxyanthraquinone-beta-quinolin.

Discovered and patented.—1881.

Shade.—Blue. Not offered.

FAVORABLE.

1. Permitted by law of Austria.

UNFAVORABLE.

1. **Chlopin** (*p. 171*): On authority not given reports this color as poisonous or harmful.
2. **Ehrlich** (*Das Sauerstoffbeduerfniss des Organismus, 1885, p. 23*): "Per kilogram of rabbit, 12–15 cc of this solution in general produce death within the first quarter of an hour; whereas 4 cc of the same did not usually produce it, and 7 cc represent a medium, when properly applied, fatal dose." (This solution contained not to exceed 17 per cent coloring matter; each cubic centimeter represents 170 milligrams per kilogram body weight, or 119 grains per 100 pounds; the coloring matter was introduced subcutaneously.)
3. **Buss** lists it as poisonous.

G. T. 572.

Trade name.—Indophenol white; Leucindophenol.

Scientific name.—Tin compound of dimethyl-para-amido-phenyl-para-oxy-alphanaphthylamin.

Discovered.—1881.

Shade.—Blue. Not offered.

FAVORABLE.

Nothing.

UNFAVORABLE.

1. Santori (*Moleschott's Untersuchungen, 1895, v. 15, p. 57*): A dog weighing 4,000 grams received 18 grams dye in 30 days, which amounts to 150 milligrams per kilo per day or 105 grains per 100 pounds per day. Temperature, urine, and weight all remained unchanged. Animal killed with chloroform; autopsy showed fatty degeneration of the liver; everything else normal. (Santori classes this color as "not nonpoisonous.")

G. T. 574.

Trade names.—Ursol D; Ursol P; Ursol DD.

Scientific name.—Hydrochlorids of para-phenylene diamin, para-amidophenol, and diamidodiphenylamin, respectively.

Discovered.—1888.

Shade.—Brown to black. Not offered.

FAVORABLE.

Nothing.

UNFAVORABLE.

1. Chlopin (*p. 214*) examined this color, and on his own experiments reports it as "strongly poisonous." On January 15, 1901, a dog weighing 18.4 kilos was given 3 grams. An hour or an hour and a half after giving dye vomiting set in; dog lay down on one side and died in 3 or 4 hours. Cause of death, heart filled with coagulated blood; lungs, liver, and kidneys filled with blood; turbid swelling of the liver and heart; mucous membrane of stomach inflamed; brain unchanged. Death caused by paralysis of the heart. It also acts severely on the skin.

G. T. 576.

Trade names.—New Gray; Malta Gray; Nigrosin; Direct Gray; Methylene Gray; New Methylene Gray.

Scientific name.—(?)

Discovered.—1888.

Shade.—Gray. Not offered.

FAVORABLE.

1. Chlopin (*p. 209*) examined this color, and on his own experiments concludes it contains "no poisonous properties." The experimental data follow:

Experimental data by Chlopin.

[1 gram=77 mg=54 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1902.	*Grams.*	*Kilos.*	*cc.*	
Feb. 8	3	12.9	510	Dog quite well; urine quite normal.
9	....	....	480	Urine green color; acid; no albumen.
10	3	....	400	Do.
11	3	....	450	Do.
12	3	....	475	Do.
13	3	....	....	Vomited once; urine dark green; acid; no albumen.
14	3	....	....	Do.
15	3	12.8	490	Do.
16	....	....	....	Urine lighter shade.
17	....	....	475	Urine normal; dog well.
Total.	21			

G. T. 584.

Trade names.—Safranin; Safranin S; Safranin conc.; Safranin GOO; Safranin T; Safranin extra G; Safranin FF extra; Safranin AG, AGT, and OOF.

Name under which it was offered on the United States market as a food color in 1907.—Safranin SP.

Scientific name.—Mixture of diamido-phenyl-and-tolyl-tolazonium chlorids.

Discovered and patented.—1859.

Shade.—Reddish brown. Offered by 1 out of 12 sources.

FAVORABLE.

1. Cazeneuve (*Arch. gén. de méd. 1886, Vol. I, p. 753*): Produces gastro-intestinal disturbances but is not a violent poison.

UNFAVORABLE.

1. Prohibited by Confectioners' List.
2. Weyl (*p. 31*): "Cazeneuve and Lépine pointed out the poisonous nature of * * * Safranine."
3. "This body (Chamber of Commerce of Sonneberg) recommends for the preparation of children's toys three colors, the poisonous character of which I can demonstrate. These are * * * Safranine * * *. (*p. 34.*)
4. Weyl (*Handbuch der Hygiene*): "According to Theodore Weyl this is even in small doses, when injected subcutaneously, a strong poison" (50 milligrams per kilo body weight, or 35 grains per 100 pounds); "whereas, when administered by the stomach only large doses over a long period of time produce diarrhea."
5. Lewin (*Lehrbuch der Toxikologie, 1897, p. 232*): "Safranin is poisonous when injected intravenously. (Pulse acceleration, dyspnœa, cramps.) Fed to dogs by the mouth it causes only diarrhea."
6. Prohibited by the Resolutions of the Society of Swiss Analytical Chemists, of September, 1891.
7. Prohibited by the Canton of Tessin.
8. Buss lists it as poisonous.
9. Cazeneuve and Lépine (*Compt. rend. 1885, v. 101, p. 1011*): A dog (weight not given) was given, by the mouth, daily doses of from 1 to 4 grams for several weeks; only salivation and diarrhea were produced. They conclude that it is a harmful color.

DOUBTFUL.

1. **Winogradow** (*Zts. Nahr. Genussm., 1903, v. 6, p. 589*) says it almost completely inhibits digestion.

G. T. 593.

Trade names.—Mauve; Mauvein; Chrome Violet.

Obsolete names.—Mauve Dye; Anilein; Anilin Purple; Violein; Indisin.

Scientific name.—Salts of phenyl- and tolyl-safranins.

Discovered and patented.—1856.

Shade.—Blue, Reddish Blue, Bluish Violet. Not offered.

FAVORABLE.

1. Permitted by Confectioners' List.

G. T. 599.

Trade names.—Indulin, spirit soluble; Indulin opal; Fast Blue R and B, spirit soluble; Indulin 3B and 6B, spirit soluble; Indulin 3B opal; Indulin 6B opal; Azin Blue, spirit soluble; Indigen D and F; Printing Blue; Acetin Blue.

Scientific name.—Mixtures of dianilidoamido, trianilido and tetraanilido phenyl-phenazonium chlorids.

Discovered and patented.—1863.

Shade.—Blue. Not offered.

FAVORABLE.

1. **Santori** (*Moleschott's Untersuchungen, 1895*, v. *15*, *p. 50*): A dog weighing 4,600 grams received 12.5 grams of this dye (in oil) in 30 days, which amounts to 91 milligrams per kilo per day, or 64 grains per 100 pounds per day. There was no disturbance of any kind. Killed by chloroform; autopsy showed everything to be normal.

G. T. 600.

Trade names.—Nigrosin, spirit soluble; Coupier's Blue; Oil Black; Sloelin; Spirit Black.

Scientific name.—Mixtures of Indulins with allied bases and fluorindins.

Discovered and patented.—1867.

Shade.—Black. Not offered.

FAVORABLE.

1. Permitted by Confectioners' List.

G. T. 601.

Trade names.—Indulin, soluble; Indulin 3B; Fast Blue R and 3R; Sloelin R S and B S; Indulin R and B; Indulin 6 B; Fast Blue 2R, B, and 6B.

Scientific name.—Mixtures of the sodium salts of the sulphonic acids of the various spirit-soluble indulins.

Discovered and patented.—1867.

Shade.—Bronzy or Blue Black. Offered by 1 out of 12 sources.

FAVORABLE.

1. CHLOPIN (*pp. 198–9*): On his own experiments classifies this color as nonpoisonous. The experimental data are as follows:

Experimental data by Chlopin.

No. 1.

[1 gram=54 mg=38 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1902.	*Grams.*	*Kilos.*	*cc.*	
Jan. 5	3	18.4	650	Dog and urine normal.
6			670	Urine brown; acid; no albumen.
7	3		400	Urine greenish brown; no albumen.
8	3		410	Do.
9	3		400	Do.
10	3		400	Do.
11	3		500	Do.
12			560	Do.
13			650	Do.
14			620	Color and composition of urine normal; dog is well.
Total.	18			

No. 2.

[1 gram=114 mg=80 grains.]

1902.				
Jan. 14	3	8.8	420	Dog and urine normal.
15	3		390	Urine slightly blue; acid; no albumen.
16	3			Do.
18			420	Do.
19				Urine almost normal color; no albumen.
20				Color and other properties of urine normal.
21	3	8.8		No record.
22	3		400	Urine slightly bluish; no albumen.
23	3		460	Do.
24			400	Dark green; no albumen.
25			450	Feeble green; no albumen.
26			400	Urine normal; dog well.
Total.	18			

UNFAVORABLE.

1. SANTORI (*Moleschott's Untersuchungen, 1895, v. 15, p. 55*): A dog weighing 4,500 grams received 4 to 6 grams dye in 7 days, which amounts to 127 to 190 milligrams per kilo per day, or 89 to 133 grains per 100 pounds per day. No vomiting. Stool black blue and no change in the urine. Up to the sixth day the animal, in very good general condition, ate heartily and was quite lively; temperature unchanged. On the morning of the sixth day the animal was found in his cage suffering from general muscular cramps which were heightened by the slightest noise; the animal did not respond to calls or threatening movements and was in a complete stupor. This continued for 24 hours, when the animal died. The autopsy showed numerous punctures of the lungs and of the mucous membrane of the stomach; fatty degeneration of the liver and little blood in it; kidneys without change, although the cortex was colored a light green.

G. T. 602.

Trade names.—Nigrosin, soluble; Bengal Blue; Gray R and B.

Scientific name.—Sodium salts of sulphonic acids of spirit nigrosins.

Discovered and patented.—1867.

Shade.—Black. Not offered.

FAVORABLE.

Nothing.

UNFAVORABLE.

1. **Lewin** (*Lehrbuch der Toxikologie, 1897, p. 231*) says: "Produces eczema," and cites *Deutsche. Med. Wochenschr., 1891, page 45.*

G. T. 614.

Trade names.—Magdala Red; Naphthalene Rose; Sudan Red; Naphthalene Red; Naphthylamin Pink.

Scientific name.—Mixture of amido-naphthyl-naphthazonium-chlorid and diamido-naphthyl-naphthazonium chlorid.

Discovered.—1868.

Shade.—Red. Not offered.

FAVORABLE.

Nothing.

UNFAVORABLE.

1. **Chlopin** (*p. 200*) examined this color, and on his own experiments concludes it "does not belong to the poisonous list, but is not wholly harmless." The experimental data are as follows:

Experimental data by Chlopin.

No. 1.

[1 gram=91 mg=64 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1902.	*Grams.*	*Kilos.*	*cc.*	
Apr. 12	2	11	780	Before experiment dog and urine normal.
13			1,100	Urine rose-colored; acid; no albumen.
14	3		760	Do.
15			660	Urine quite red; acid; no albumen; vomited once.
18			670	No vomiting; dog is well; urine normal in color, and composition.
Total.	5			

No. 2.

[1 gram=111 mg=78 grains.]

1903.				
Feb. 3	3	9	380	Day before experiment quite well; urine normal color; acid; no albumen.
4	3		340	In the morning three times thin stool, urine faintly rose-colored; acid; no albumen.
5	3		370	No diarrhea; urine rose-red; acid; no albumen.
6				Do.
7	3			Do.
8	3		310	Do.
9			320	Do.
Total.	15			

Experimental data by Chlopin—Continued.

[1 gram=114 mg=80 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1903.	*Grams.*	*Kilos.*	*cc.*	
Feb. 10	3	....	310	Vomiting and diarrhea; urine rose-red; no albumen; acid; with muriatic acid rose color, becomes greenish gray.
11	....	....	315	No vomiting; no diarrhea; urine rose-red; acid; no albumen.
12	....	8.8	350	No vomiting; no diarrhea; color reaction of urine normal; no albumen.
Total.	3			

DOUBTFUL.

1. Winogradow (*Zts. Nahr. Genussm. 1903, v. 6, p. 589*) says it almost completely inhibits digestion.

G. T. 620.

Trade names.—Gallocyanin DH and BS; Fast violet; Gallocyanin RS, BS, and D.

Scientific name.—Dimethylamido dioxy phenazoxoniumcarboxylate. (BS is the bisulphite compound.)

Discovered and patented.—1881.

Shade.—Bluish Violet. Not offered.

FAVORABLE.

Nothing.

UNFAVORABLE.

1. Santori (*Moleschott's Untersuchungen, 1895, v. 15, p. 51*): A dog weighing 5,400 grams received 7.5 grams dye in 30 days, which amounts to 46 milligrams per kilo per day or 32 grains per 100 pounds per day. Throughout all the time the animal remained well, had good appetite, temperature normal, no loss of weight; urine and feces colored deep blue black. Killed by chloroform. Autopsy showed incipient fatty degeneration of the liver and a swelling of the kidneys. Conclusion: Poisonous.

G. T. 639.

Trade names.—Meldola's Blue; Cotton Blue R; Fast Navy Blue R; Naphthol Blue R and D; Naphthylene Blue R in crystals; Fast Blue R, 2 R and 3 R for cotton in crystals; Fast Navy Blue RM and MM.

Scientific name.—Zinc double chlorid of dimethylamido-naphthophenoxazonium chlorid.

Discovered.—1879.

Shade.—Dark Violet. Not offered.

FAVORABLE.

Nothing.

UNFAVORABLE.

1. Chlopin (*pp. 194–195*) on his own experiments reports this color as "very poisonous." The experimental data are as follows:

Experimental data by Chlopin.

No. 1.

[1 gram=68 mg=48 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1902. Jan. 5...	*Grams.* 3	*Kilos.* 14.6	*cc.* 590	Before experiment dog quite well; urine normal in color and composition; 1 hour after giving the dye vomiting began; dog lay down on ground and died in 1 hour.
Total.	3			

No. 2.

[1 gram=167 mg=117 grains.]

1902. Jan. 14	3	6	....	Soon after giving dye, strong vomiting and diarrhea; after a few hours improved.
15	3	....	....	Most violent vomiting; dog lay down on his side, and was found dead in 6 hours.
Total.	6			

NOTE.—See p. 181.

2. SANTORI (*Moleschott's Untersuchungen, 1895, v. 15, p. 49*): A dog weighing 4,500 grams received 12.5 grams dye during 30 days; this amounts to 93.3 milligrams per kilo per day or 65.3 grains per hundred pounds per day. Continued vomiting beginning with 0.2 grams dye, anaemia and copious as well as continuous salivation and emaciation; the animal lost 1,200 grams in weight or 27 per cent. Killed by chloroform; autopsy showed no fat, flabby muscles, stomach contracted and filled with mucous and in part colored pea green; fatty degeneration of the liver; contracted bladder; kidneys swollen and congested with blood and decomposed blood corpuscles in the Bowman capsules.

G. T. 649.

Trade names.—Gentianin; Gentiana Violet.

Scientific name.—Zinc double chlorids of dimethyldiamido phenazthionium chlorid.

Discovered and patented.—1886.

Shade.—Violet. Not offered.

FAVORABLE.

Nothing.

UNFAVORABLE.

1. SANTORI (*Moleschott's Untersuchungen, 1895, v. 15, p. 54*): A dog weighing 3,000 grams received 4.7 grams of dye in 7 days, which amounts to 224 milligrams per kilo per day or 157 grains per 100 pounds per day. Beginning with the third day the dog appeared weak and depressed and a whitish froth appeared at the mouth; mild diarrhea and complete aversion to food; temperature and urine unchanged. Died on seventh day. Autopsy showed congestion of mucous membrane of stomach; the liver was inflamed and the kidneys strongly congested.

G. T. 650.

Trade names.—Methylene Blue B and BG; Methylene Blue BB in powder extra D; Methylene Blue BB in powder extra; Methylene Blue A extra.

Names under which it was offered on the United States market as a food color in 1907.—Methylene Blue B; Methylene Blue.

Scientific name.—Chlorid or zinc double-chlorid of tetramethyl-diamido-phenazthionium.

Discovered.—1876.

Shade.—Blue. Offered by 2 out of 12 sources.

FAVORABLE.

1. **Schacherl** (*p. 1046*): "To these groups belong the much-used Methylene Blue, which in moderate doses is harmless."
2. **Fraenkel** (*p. 574*): "* * * Methylene Blue causes no noteworthy disturbances."
3. **Cazeneuve** (*Arch. gén. de. méd. 1886, v. 1, p. 753*) says that it produces gastric intestinal derangements but is not a violent poison.

UNFAVORABLE.

1. Prohibited by Confectioners' List.
2. **Weyl** (*p. 31*): "Cazeneuve and Lépine pointed out the poisonous nature of * * * Methylene Blue * * *."
3. **Fraenkel** (*p. 579*): "To regard Methylene Blue as a specific remedy such as quinin, is, in spite of a few such experiments, improper; it produces subsidiary effects which depend in part upon local irritation of the intestinal tract, and partly, however, upon irritation of the bladder with increased micturition."
4. **Cazeneuve** (*Arch. gén. de méd. 1886, v. 1, p. 753*), says that it produces gastric intestinal derangements.
5. **Combemale** and **Francois** (*Sem. Med. 1890, no. 31, p. 258*), say that it produces intestinal disorders and vomiting, colored urine, and colored feces in dogs, and therefore is a highly injurious color.
6. **Santori** (*Moleschott's Untersuchungen, 1895, v. 15, p. 42*) classes it as injurious. A dog weighing 4,600 grams received in 20 days 18 grams dye, which amounts to 196 milligrams per kilo per day, or 137 grains per 100 pounds per day. Urine and feces colored; diarrhea and continuous vomiting; blood and pus contained in stool; loss of appetite; loss of weight was 1,600 grams, or 35 per cent. Animal died. Autopsy disclosed blue-colored skin and fat; brain turned blue on exposure to air, but only the outer cortex was colored; stomachical catarrh; the mucous membrane of the stomach colored blue; the heart sac and the pleura colored blue to blue-green; intestines externally blue; kidneys were thickened and colored dark blue throughout; parenchymatous kidney inflammation; fatty degeneration of the liver; diaphragm locally colored.
7. **Arloing** and **Cazeneuve**, quoting Cazeneuve and Lépine (*Arch. de méd. v. 9, p. 364*), say that it is not inactive.
8. Forbidden by Resolutions of Swiss Analytical Chemists, September, 1891.

9. **Galliard** (*Rev. intern. des falsifications; abst. Hygien. Runsdach. 1892, p. 104*): "Methylene Blue, which is frequently used for coloring foods, can cause, even in small doses (10 to 20 milligrams, or one-sixth to one-third of a grain), a feeling of general depression, nausea, and certain feelings of pain, and can even produce transitory albuminuria. In larger doses (40 to 60 milligrams, or six-tenths to nine-tenths of a grain), it causes in the case of persons not accustomed thereto, vomiting, diarrhea, increased micturition, and albuminuria. In the case of persons suffering from nervous diseases, it frequently produces disturbance which has as its consequence a cessation or a change of place of the pain. Sometimes it produces painlessness, or an easing of pain in the patient, for which no certain therapeutic indication could be determined."
10. **Weyl** (*Handbuch der Hygiene*) comments as follows: "These statements of Galliard arouse but little confidence, because, as is well known, Methylene Blue is very frequently administered to invalids in doses of more than 0.5 gram without any noticeable disturbance. Perhaps Galliard's preparation was unclean. At any rate, in all experiments on the poisonous nature of Methylene Blue it is to be considered that it frequently occurs in commerce as a zinc chlorid double salt."
11. **Lewin** (*Lehrbuch der Toxikologie, 1897, p. 232*): "Methylene Blue * * * can produce after prolonged administration of 0.5–1.5 gram daily increased micturition, irritation of the bladder, blue coloration of the urine, and saliva, diarrhea, headaches, vertigo, delirium, and twitching of the muscles, the latter symptoms probably because the coloring matter is deposited in the brain."
12. **Buss** lists it as poisonous.

G. T. 651.

Trade name.—Methylene Green G conc. extra yellow shade.
Scientific name.—Nitromethylene Blue.
Discovered and patented.—1886.
Shade.—Green. Not offered.

FAVORABLE.

Nothing.

UNFAVORABLE.

1. **Chlopin** (*pp. 192–193*) examined this color, and on his own experiments concludes that it is "nonpoisonous, but somewhat suspicious." The experimental data are as follows:

Experimental data by Chlopin.

No. 1.

[1 gram=71 mg=50 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1901.	*Grams.*	*Kilos.*	*cc.*	
Apr. 24	2	14	580	Dog well; urine normal.
25				Urine dark green; acid; no albumen.
26				Urine less colored; acid; traces of albumen.
28	2			No record.
29				Urine dark brown; no albumen; acid.
30			485	Do.
May 2				Color normal; acid; no albumen.
Total.	4			

Experimental data by Chlopin—Continued.

No. 2.

[1 gram=122 mg=85 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1901.	*Grams.*	*Kilos.*	cc.	
Nov. 25	3	8.2	430	Dog well; urine normal.
26	3	....	445	Urine strongly green; no albumen.
27	3	....	450	Do.
28	3	....	410	Diarrhea; urine green; acid; no albumen.
29	3	....	440	No diarrhea; urine same.
Dec. 1-2	....	....	....	Color normal; acid; no albumen; dog lively and well.
Total.	15			

DOUBTFUL.

1. **Winogradow** (*Zts. Nahr. Genussm., 1903, v. 6, p, 589*), says it noticeably retards digestive action; is not indifferent.

G. T. 654.

Trade name.—Toluidin Blue O.

Scientific name.—Zinc-double-chlorid of dimethyl-diamido-tolu-phenazthonium-chlorid.

Discovered and patented.—1888.

Shade.—Blue. Not offered.

FAVORABLE.

Nothing.

UNFAVORABLE.

1. **Fraenkel** (*p. 574*): "Toluidin Blue * * * is a strong poison for micro-organisms, and may be used in eye treatment, like Methylene Blue."

G. T. 659.

Trade names.—Primulin; Thiochromogen; Sulphin; Polychromin; Aureolin.

Scientific name.—Sodium salt of the mono-sulphonic acids of the dehydrothionated condensation products of dehydrothiotoluidin (mixed with some sodium dehydrothiotoluidin-sulphonate).

Discovered.—1887.

Shade.—Yellow. Not offered.

FAVORABLE.

Nothing.

UNFAVORABLE.

1. **Chlopin** (*p. 203*) examined this color, and on his own experiments reports it as "suspicious." The experimental data are as follows:

Experimental data by Chlopin.

[1 gram=154 mg=108 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1902.	*Grams.*	*Kilos.*	*cc.*	
Mar. 21	2.0	6.5	292	Dog and urine normal.
22			294	Urine dark brown; insignificant traces of albumen; acid.
23	3.0		355	Color normal; no albumen.
24	[1] 0.5	6.3	260	Urine dark brown; acid; no albumen.
25				Do.
26				No albumen; dark-brown color; dog has lassitude; does not eat bread nor take milk, only a little meat.
27			291	No albumen; dark-brown color; dog has lassitude; dog eats little.
28				At the point of injection an abscess appears; urine acid; no albumen; yellow color.
29			270	Urine acid; no albumen; yellow color; dog eats more.
30			295	Urine acid; no albumen; yellow color; abscess broke.
Apr. 2			280	Urine normal; appetite almost normal.
Total	[1] 5.0 [2] 0.5			

[1] By mouth.

[2] Subcutaneously.

2. See also CHLOPIN (*Zts. Nahr. Genussm., 1902, v. 5, p. 241*).

DOUBTFUL.

1. WINOGRADOW (*Zts. Nahr. Genussm., 1903, v. 6, p. 589*) says it noticeably retards digestive action; is not indifferent.

G. T. 667.

Trade names.—Quinolin Yellow; Quinolin Yellow, water-soluble.

Name under which it was offered on the United States market as a food color in 1907.—Chinolin Yellow O.

Scientific name.—Sodium salt of the sulphonic acid (chiefly disulphonic acid) of quinophthalone.

Discovered.—1882.

Shade.—Greenish Yellow. Offered by 1 out of 12 sources.

FAVORABLE.

Nothing.

UNFAVORABLE.

1. CHLOPIN (*p. 205*) on his own experiments reports this color as "suspicious." The experimental data are as follows:

Experimental data by Chlopin.

No. 1.

[1 gram=44 mg=31 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1902.	*Grams.*	*Kilos.*	*cc.*	
Mar. 19	3.00	22.6	730	Before experiment dog and urine normal.
20			720	Urine dark brown; traces albumen; acid.
21			749	Color of urine normal; no albumen.
22				Do.

Experimental data by Chlopin—Continued.

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1902.	*Grams.*	*Kilos.*	*cc.*	
Mar. 23	0.75	21.4	750	Urine dark brown; no albumen.
24				Dog eats nothing.
25			693	Do.
26			725	Do.
27				Dog eats nothing; eats poorly; albumen in urine.
28				Dog eats nothing; rather much albumen.
29				Do.
30				Do.
Apr. 2				Dog eats nothing; traces of albumen.
4	[1] 3.00		710	Color of urine almost normal; insignificant traces of albumen.
5				Urine yellow brown; traces of albumen; appetite normal.
6				Urine yellow brown; no albumen.
8			700	Urine and dog normal.
Total..	[1] 6.00 [2] 0.75			

No. 2.

[1 gram=133 mg=83 grains.]

1903.				
Mar. 3	3.00	7.5	320	Before experiment dog quite well, and urine normal.
4	3.00		300	Color dark yellow; no albumen; general condition normal.
5	3.00		300	Color yellower than normal; acid; no albumen.
6	2.00	7.4		Do.
Total..	11.00			

[1] By mouth.

[2] Subcutaneously.

DOUBTFUL.

1. **Winogradow** (*Zts. Nahr. Genussm., 1903, v. 6, p. 589*) says it noticeably retards digestive action; is not indifferent.

G. T. 670.

Trade names.—Vidal Black; Vidal Black S.

Scientific name.—Possibly the sulpho-hydro derivative of a polythiazin.

Discovered.—1893.

Shade.—Green. Not offered.

FAVORABLE.

Nothing.

UNFAVORABLE.

1. **Chlopin** (*p. 208*) examined this color, and on his own experiments classes it as "very harmful." The experimental data are as follows:

Experimental data by Chlopin.

[1 gram=132 mg=92 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1901.	*Grams.*	*Kilos.*	*cc.*	
Dec. 29	2	7.6	386	Before experiment dog quite normal; soon after taking dye vomited twice.
30	3		390	General condition good; no vomiting; urine turbid, alkaline; no albumen.

Experimental data by Chlopin—Continued.

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1901.	*Grams.*	*Kilos.*	*cc.*	
Dec. 31	3	....	....	Vomited soon after getting dye; appetite less; no albumen.
1902.				
Jan. 1	3	....	....	Urine turbid; scarcely noticeable blackish sheen; green with sulphuric and hydrochloric acid.
2	....	7	369	Do.
4	3	....	370	Vomited all day after taking dye; eats little; no albumen.
5	3	....	....	Violent and prolonged vomiting and diarrhea; condition pitiable; further giving of dye stopped, so as not to kill the animal.
6–7	....	....	....	Vomiting and diarrhea continue.
8	....	....	....	Vomiting and diarrhea stopped.
9–11	....	....	....	Dog is livelier, and begins to eat.
13	....	....	....	Dog looks well; urine normal in color; acid; no albumen.
Total..	17			

G. T. 675.

Trade names.—Thiocatechin; Thiocatechin S.
Shade.—Brown. Not offered.

FAVORABLE.

Nothing.

UNFAVORABLE.

1. Chlopin (*pp. 210, 211*) examined this color and classes it as "very poisonous." The experimental data are as follows:

Experimental data by Chlopin.

[1 gram=128 mg—90 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1902.	*Grams.*	*Kilos.*	*cc.*	
Feb. 13	2.4	7.8	300	Before experiment dog and urine quite normal; 10 or 15 minutes after giving dye dog fell on one side, limbs extended, stomach drawn in; small and frequent convulsions; retching; then abundant vomiting, same color as dye; soon after vomiting dog got up and walked as if drunk; hind legs tend to collapse; salivation.
14	2.0	....	290	A few minutes after giving dye, dog again fell as if in an epileptic fit; convulsions of the extremities, which soon ceased, but the dog still lay stretched out, with open eyes, which reacted to light; tongue hanging out to one side; after 10 or 15 minutes vomiting began; dog still lying on one side, assumed a more normal attitude; 15 minutes later dog got up, walked as if drunk; poor control over hind legs.
15	0.5	....	....	Vomits at once after getting dye, but remained standing.
16	....	....	....	Dog appears depressed, but eats.
18	....	....	....	Dog quite well; urine usual normal color; no albumen.
Total..	4.9			

G. T. 689.

Trade names.—Indigo; Indigo Pure BASF.
Scientific name.—Indigotin.
Shade.—Blue. Not offered.

FAVORABLE.

1. Permitted by Confectioners' List.
2. Permitted by the law of Italy.

UNFAVORABLE.

1. FRAENKEL (*p. 571*): "However, pure Indigo, according to Kobert, is, in finely divided condition, a violent local irritant."

G. T. 692.

Trade names.—Indigo Carmine; Indigo Extract; Indigotin.

Names under which it was offered on the United States market as a food color in 1907.—Indigo Carmine Powder IN; Indigotin; Indigotin A.

Scientific name.—Sodium salt of Indigotin disulphonic acid or the free acid.

Discovered.—1740.

Shade.—Blue. Offered by 3 out of 12 sources.

FAVORABLE.

1. SCHACHERL (*p. 1046*): "No objection to its use."
2. SANTORI (*Moleschott's Untersuchungen, 1895, v. 15, p. 41*): A dog weighing 4,500 grams received 90 grams dye in 30 days; vomited twice during the examination; no change in weight; animal killed with chloroform; autopsy showed slight dull swelling in the epithelium and convoluted canals of the kidneys. This dosage amounts to 667 milligrams per kilo per day, or 467 grains per 100 pounds per day. Classes it as harmless.

ALPHABETICAL INDEX OF TRADE NAMES OF COAL-TAR COLORS.

The following list of the trade names of coal-tar colors appearing in the foregoing compilation on physiological action is complete when supplemented by the list of 23 colors given on page 227; Green Table numbers in parenthesis.

Acetin Blue (599).
Acid Brown (138).
Acid Green (434, 435).
Acid Green ex. conc. (435).
Acid Green conc. (435).
Acid Green conc. VN (435).
Acid Green conc. 780 (435).
Acid Fuchsin (462).
Acid Magenta (462).
Acid Magenta powd. (462).
Acid Rosein (462).
Acid Rubin (462).
Acid Violet (467).
Acid Violet 6 B (467).
Acid Yellow (8).
Acid Yellow AT (94).
Acid Yellow D (88).
Acid Yellow G (8).
Acid Yellow G pat. (8).
Acme Yellow (84).
Alizarin Blue ABS (563).
Alizarin Blue S (563).
Alizarin Green (527).
Alphanaphthol Orange (85).
Alsace Green (394).
Amaranth (107).
Amaranth B (107).
Anilein (593).
Anilin Blue Sp. Sol. (457).
Anilin Orange (2).
Anilin Purple (593).
Anilin Red (448).
Anilin Yellow (4).
Anilin Yellow S (4).

Anthracene Green (527).
Anthracene Blue S (563).
Archil Substitute V (28).
Atlas Orange (86).
Auramin (425).
Auramin O (425).
Aurantia (6).
Aureolin (659).
Aurin (483).
Azalein (408).
Azarin S (70).
Azin Blue, spirit soluble (599).
Azo Acid Rubin (103).
Azo Acid Yellow (92).
Azo Blue (287).
Azo Flavin (92).
Azo Fuchsin G (93).
Azo Rubin (103).
Azo Rubin A (103).
Azo Rubin S (103).
Bavarian Blue DBF (479).
Bavarian Blue DSF (478).
Bengal Blue (602).
Bengal Green (427).
Benzoflavin (530).
Benzopurpurin 4B (277).
Betanaphthol Orange (86).
Biebrich Scarlet (163).
Bismarck Brown (197).
Bismarck Brown R (201).
Bitter-almond-oil Green (427).
Bleu de Nuit (457).
Bleu Lumière (457).
Bleu Marine (480).
Bordeaux B (65).
Bordeaux B L (65).
Bordeaux R ext. (65).
Bordeaux S (107).
Brilliant Black B (188).
Brillant Cotton Blue, greenish (479).
Brilliant Green (428).
Brilliant Orange (13).
Brilliant Scarlet (106).
Brilliant Scarlet 4R (106).
Brilliant Yellow (5).
Brilliant Yellow S (89).
Butter Yellow (16).
Canary Yellow (425).
Carbazotic Acid (1).
Cardinal 3B (103).
Carminaph (11).
Carmoisin (103).
Carmoisin B (103).
Cerasin (102).
Cerasin Orange I (11).
China Blue (480).
Chlorin (394).
Chrome Violet (593).
Chrysamin R (269).
Chrysaurein (86).
Chryseolin (84).
Chrysoidin Crystals (17, 18, 41).
Chrysoidin R (17, 18, 41).
Chrysoidin Y (17, 18, 41).
Chrysoin (84).
Chrysoin REZ (84).
Cinnamon Brown (197).
Citronin (4).
Claret Red (65).
Claret Red RZ (103, 106, 107).
Cœrulein S (527).
Cochineal Red A (106).
Congo Red (240).
Cotton Blue (480).
Cotton Blue R (639).
Cotton Red 4B (277).
Coupier's Blue (600).
Crocein Orange (13).
Crocein Orange 10234 (13).
Crocein Orange G (13).
Crocein Scarlet (106).
Crocein Scarlet 3B (160).
Crocein Scarlet 4BX (106).
Crocein Scarlet 7B (169).
Crocein Scarlet 8B (169).
Curcumin (89).
Curcumin S (399).
Dahlia (450, 451).
Dark Green (394).
Diamond Green B (427).
Dianthin G (516).
Dinitro-cresol (2).
Dinitrosoresorcin (394).
Diphenylamin Blue (479).
Diphenylamin orange (88).
Direct Gray (576).
Direct Violet (451).
Emerald Green (428).
Emerald Green cryst. (428).
English Brown (197).
Eosin (512).
Eosin A (512).
Eosin A ex. (512).
Eosin B (512).
Eosin bluish (517).
Eosin G G F (512).

Eosin J (512, 517).
Eosin 10 B (521).
Eosin 3J & 4J ext. (512).
Eosin Y (512).
Eosin Yellowish (512).
Erika B (78).
Erythrobenzin (448).
Erythrosin (517).
Erythrosin B (517).
Erythrosin D (517).
Erythrosin G (516).
Erythrosin yellow shade (517).
Ethyl Green (428).
Fast Blue (477).
Fast Blue B, spirit soluble (599).
Fast Blue R and 3R (601).
Fast Blue R, spirit soluble (599).
Fast Blue 2R, B, and 6B (601).
Fast Blue R, 2R, and 3R (639).
Fast Brown G (138).
Fast Green (427).
Fast Green J (428).
Fast Green O (394).
Fast Myrtle Green (394).
Fast Navy Blue R (639).
Fast Navy Blue RM and MM (639).
Fast Ponceau B (163).
Fast Red (102, 105).
Fast Red C (103).
Fast Red D (107).
Fast Red EB (107).
Fast Violet (620).
Fast Yellow (4, 8, 9, 88).
Fast Yellow extra (8).
Fast Yellow G (8).
Fast Yellow 053 (8).
Fast Yellow R (9).
Fine Blue (457).
Fuchsiacin (448).
Fuchsin (448).
Fuchsin cryst. (448).
Fuchsin S (462).
Gallocyanin DH and BS (620).
Gallocyanin RS, BS, and D (620).
Gentian Blue 6B (457).
Gentiana Violet (649).
Gentianin (649).
Gold Orange (86).
Gold Yellow (84).
Golden Yellow (2, 3).
Gray R and B (602).
Green E (428).
Green 087 (428).
Guinea Green (433).
Harmalin (448).
Helianthin (87).
Helvetia Blue (479).
Hessian Blue (457).
Hofmann Violet (450).
Hydrazin Yellow (94).
Imperial Scarlet (163).
Imperial Yellow (6).
Indigen D and F (599).
Indigo (689).
Indigo Carmine (692).
Indigo Carmine powd. IN (692).
Indigo extract (692).
Indigo pure BASF (689).
Indigotin (692).
Indigotin A (692).
Indisin (593).
Indisin R and B (601).
Indophenol (572).
Indophenol white (572).
Indulin opal. (599).
Indulin sol. (601).
Indulin, spirit soluble (599).
Indulin 3B opal. (599).
Indulin 6B opal. (599).
Indulin 3B sp. sol. (599).
Indulin 6B sp. sol. (599).
Indulin 3B (601).
Indulin 6B (601).
Iodeosin B (517).
Iodeosin G (516).
Iodin Green (459).
Iodin Violet (450).
Jaune Acide (4, 8).
Jaune Acide C (4).
Jaune Naphthol (3).
Jaune d'Or (3).
Jaune d'Orient (516).
Jaune Soleil (399).
Kaiser Yellow (6).
Kermesin Orange (97).
Leather Brown (197).
Leather Yellow (532).
Lemon Yellow (4).
Light Green S F bluish (434).
Light Green S F yellow shade (435).
London Blue extra (480)
Magdala Red (614).
Magenta (448).
Magenta F A B S Red (448).
Magenta Powder A (448).
Maize (399).
Malachite Green (427).
Malachite Green B (427–428).

Malta Gray (576).
Manchester Brown (197).
Manchester Brown EE (201).
Manchester Yellow (3).
Mandarin G R (97).
Mandarin G ext. (86).
Martius Yellow (3).
Mauve (593).
Mauve Dye (593).
Mauvein (593).
Meldola's Blue (639).
Metanil Yellow (95).
Methyl Blue water soluble (478).
Methyl Blue for silk MLB (478).
Methyl Blue O (479).
Methyl Violet (451).
Methyl Violet B (451).
Methyl Violet BB ext. (451).
Methyl Violet 3 BD (451).
Methylene Blue (650).
Methylene Blue A ext. (650).
Methylene Blue B and BG (650).
Methylene Blue B D (650).
Methylene Blue BB extra (650).
Methylene Blue BB extra D (650).
Methylene Gray (576).
Methylene Green G. conc. ext. (651).
Naphthalene Pink (614).
Naphthalene Red (614).
Naphthalene Rose (614).
Naphthol Black B (188).
Naphthol Black BDF (188).
Naphthol Blue R & D (639).
Naphthol Green (398).
Naphthol Green B (398).
Naphthol Orange (85).
Naphthol Red S (107).
Naphthol Yellow (3, 4).
Naphthol Yellow L (4).
Naphthol Yellow S (4, 5).
Naphthol Yellow SLOZ (4, 86).
Naphthylamin Yellow (3).
Naphthylene Yellow (2).
Navy Blue B (478).
New Coccin (106).
New Gray (576).
New Green (427).
New Methylene Gray (576).
New Red L (163).
New Victoria Green (427, 428).
New Yellow (88).
New Yellow L (8).
Nicholson Blue (477).
Night Green (459).
Nigrosin (576).
Nigrosin sol. (602).
Nigrosin sp. sol. (600).
Nitrodiphenylamin (6).
Nitromethylene Blue (651).
Oil Black (600).
Oil Orange 7078 (11).
Oil Yellow (16).
Opal Blue (457).
Orange (86).
Orange I (85).
Orange II (86).
Orange III (87).
Orange IV (88).
Orange A (86).
Orange A extra (86).
Orange Brown (17, 18, 41).
Orange A 1201 (86).
Orange B (85).
Orange extra (86).
Orange G (14).
Orange GG (14).
Orange GG Crystals (14).
Orange GRX (13).
Orange GS (88).
Orange GT (43).
Orange M (88).
Orange MN (95).
Orange N (43,88).
Orange O 27 (85).
Orange O (43).
Orange R (15, 55, 97).
Orange RN (43).
Orange RZ (85).
Orange T (97).
Orange 2 R (97).
Orange Y (86).
Orcellin No. 4 (102).
Paris Violet (451).
Phenylene Brown (197).
Philadelphia Yellow G (532).
Phloxin (521).
Phloxin T A (521).
Phosphin (532).
Picric Acid (1).
Pistachio (435).
Polychromin (659).
Pomona Green (459).
Ponceau B (163).
Ponceau 4 GB (13).
Ponceau 4 RB (160).
Ponceau G and GR (55).
Ponceau R (55).
Ponceau 3 RB (163).

Ponceau 2 G (15).
Ponceau 2 R (55).
Ponceau 6 RB (169).
Primula (450).
Primulin (659).
Printing Blue (599).
Pure Soluble Blue (480).
Pyoctanin Aureum (425).
Pyoctanin (451).
Pyrosin B (517).
Pyrosin J (516).
Quinolin Yellow, water-soluble (667).
Quinolin Yellow (667).
Rauracienne (102).
Red (107).
Red Violet 5 R extra (450).
Resorcin Yellow (84).
Resorcein O 275 (84).
Rhodamin (504).
Rhodamin B (504).
Rhodamin O (504).
Rhodamin B extra (504).
Rhodamin G and G extra (502).
Roccellin (102).
Rose Bengal (520).
Rose Bengal AT (520).
Rose Bengal G (520).
Rose Bengal N (520).
Rosein (448).
Rosolic acid (483).
Rubianite (448).
Rubidin (102).
Rubin (448).
Rubin S (462).
Russian Green (394).
Saffron Substitute (2).
Saffron Yellow (3, 4).
Safranilin (504).
Safranin (584).
Safranin AG, AGT, and OOF. (584).
Safranin Conc. (584).
Safranin extra G (584).
Safranin FF extra (584).
Safranin GOO. (584).
Safranin S. (584).
Safranin SP. (584).
Safranin T. (584).
Scarlet (55).
Scarlet L. (106).
Sloelin (600).
Sloelin RS. and BS. (601).
Solferino (448).
Solid Yellow (4).
Soluble Blue (480).
Spirit Black (600).
Spirit Blue (457).
Soluble Blue 8 B. (479).
Soluble Blue 10 B. (479).
Soluble Blue XL. (479).
Succinic (4).
Sudan I (11).
Sudan Red (614).
Sulphin (659).
Sulphin Yellow (4).
Sulphonaphthol Acid Yellow (4).
Sultan Red 4 B. (277).
Sun Yellow (399).
Tartrazin (94).
Thio Catechin (675).
Thio Catechin S. (675).
Toluidin Blue O. (654).
Tropæolin D. (87).
Tropæolin G. (95).
Tropæolin O (84).
Tropæolin OO (88).
Tropæolin OOO (85).
Tropæolin OOO No. 2 (86).
Tropæolin R (84).
Ursol D. (574).
Ursol DD. (574).
Ursol P. (574).
Vert Diamant (427).
Vert Lumière (459).
Vesuvin (197).
Vesuvin B. (201).
Victoria Blue B. (488).
Victoria Blue 4 R. (490).
Victoria Orange (2).
Victoria Yellow (2).
Victoria Yellow Conc. Z. (95).
Vidal Black (670).
Vidal Black S. (670).
Violein (593).
Violet de Methylanilin (451).
Violet R. (450).
Violet RR. (450).
Violet 5 R. (450).
Water Black (166).
Water Blue 6 B extra (480).
Water Blue (480).
Xanthin (532).
XL Soluble Blue (479).
Xylidin Red (55).
Xylidin Scarlet (55).
Yellow Corallin (483).
Yellow FY. (4).
Yellow MXX Conc. (95).
Yellow W (9).
Yellow WR. (89).

X. DOSAGE AND SYMPTOMS.

CONFECTIONERS' LIST AS A BASIS FOR A RULE.

Considering the Confectioners' List of 1899 as a correct guide as to which colors are harmful and which are harmless, the attempt has been made to determine how far dosage and the corresponding physiological effects may serve as a guide in determining which colors, other than those enumerated in either portion of the Confectioners' List, are harmful or harmless. (See p. 48.)

To this end the available literature has been searched and classified, and wherever it was possible to arrive at any conclusion as to the actual dose or the average dose over a stated period of time, and the corresponding physiological observations, these data have been separated and brought together for the purpose of making comparisons and deductions therefrom.

It was thought that the literature would show that if a dog or other animal is killed by a certain given amount of color per 100 pounds of body weight of the animal that such color is always harmful; that if untoward effects, such as vomiting, diarrhea, weakness, and general depression, are caused by more than a certain weight of color per 100 pounds body weight of the animal, such color is always regarded as harmless.

The classification of the available literature and the conclusions therefrom are as follows:

Of the 33 coal-tar colors listed as harmless in the Confectioners' List, 10, namely, G. T. 4, 9, 55, 65, 85, 103, 105, 107, 448, and 462, have been tested on humans, while the conclusion as to the remaining 23 is reached by the effects observed on dogs alone. However, contradictory statements are recorded in the case of No. 9, and none of these tests was of long-continued duration, but, on the contrary, in many cases the time covered was exceedingly short, and the conclusions deduced are, therefore, not necessarily final nor correct. It should be further noted that Nos. 95 and 106, reported as nonpoisonous to humans, are in the harmful section of this Confectioners' List.

I. Those colors which produced no effect are as follows (the number of grains given is the amount administered per 100 pounds body weight; where the data permitted, the number of days' duration of the experiment is also given:

		Grains.
5.	Brilliant Yellow S	532
13.	Ponceau 4 GB	113
521.	Phloxin	100
521.	Phloxin	300

II. Those colors which in some cases produced no effect, and in others produced effects, are as follows:

4. Naphthol Yellow S:
  395 grains produced diarrhea and no albuminuria.
  292 grains produced albuminuria.
  116 grains for 25 days produced no effect.
  25 grains for 2 weeks, on alternate days, produced no effect.
8 and 9. Fast Yellow Y and R:
  173 grains for 3 weeks produced no effect.
  53 grains produced albuminuria.
55. Ponceau 2 R.
  582 grains killed.
  198 grains produced no effect.
65. Fast Red B:
  143 grains for 145 days produced no effect.
  137 grains for 145 days produced no effect.
  98 grains for 145 days produced no effect.
  68 grains produced albuminuria.
  38 grains produced albuminuria.
103. Azorubin S:
  143 grains for 145 days produced no effect.
  137 grains for 145 days produced no effect.
  98 grains for 145 days produced no effect.
  70 grains produced diarrhea.
287. Azo Blue:
  233 grains produced no effect.
  166 grains produced albuminuria.

III. Those that produced only a slight disturbance which was regarded as negligible:

28. Archil Substitute V:
  301 grains produced vomiting and albuminuria.
  127 grains produced vomiting and albuminuria.
105. Fast Red E:
  70 grains produced diarrhea.
166. Wool Black:
  117 grains produced albuminuria.
240. Congo Red:
  192 grains produced albuminuria.
269. Chrysamin R:
  433 grains produced albuminuria.
  361 grains produced albuminuria.
394. Dinitrosoresorcin:
  139 grains produced loss of appetite.
  121 grains produced albuminuria.

From Class I above it would appear—

(*a*) That if a dose of 113 grains per 100 pounds body weight produced no effect it is to be classed as harmless.

From Class II above it would appear—

(*a*) That if albuminuria is caused by as little as 38 grains in some cases, and no effect is produced by doses as large as 143 grains, the color is to be classed as harmless.

(*b*) Also that if 198 grains produce no effect the color is to be classed as harmless, even if 582 grains in another case kill the animal.

(*c*) That if small amounts produce albuminuria and larger amounts do not the color is to be classed as harmless.

(*d*) That if small amounts produce diarrhea and larger amounts do not the color is to be classed as harmless.

From Class III it would appear that—

(*a*) Albuminuria produced by as little as 117 grains is to be regarded as not harmful.

(*b*) Diarrhea produced by 70 grains is to be regarded as not harmful.

(*c*) Vomiting and albuminuria produced by 127 grains are to be regarded as not harmful.

The colors classed as harmful in the Confectioners' List may be classified as follows:

I. *Dogs.*—Those colors that under certain conditions produce no noticeable effects on dogs, while under other conditions effects are observed, are classed as harmful colors:

1. Picric Acid:
    Dogs stand 5 grains daily for 18 days without effect.
    18½ grains produced diarrhea.
    20.4 grains killed.
2. Dinitrocresol:
    140 grains caused vomiting but did not kill.
    38½ grains caused vomiting but did not kill.
    38½ grains caused vomiting and did kill.
    35 grains caused vomiting but did not kill.
    31.6 grains caused vomiting but did not kill.
3. Martius Yellow:
    51 grains do not kill, but produce weakness, diarrhea, and albuminuria.
    50 grains kill.
    8 grains do not kill, but produce weakness, diarrhea, and albuminuria.

86. Orange II:
    714 grains produced kidney irritation, thirst, and diarrhea.
    333 grains kill, and produced diarrhea and albuminuria.
    244 grains produced kidney irritation, thirst, and diarrhea.
    36 grains produced no effect.

95. Metanil Yellow:
    620 grains produce vomiting.
    603 grains kill.
    407 grains no effect.
    371 grains kill.
    104 grains produced albuminuria.
    62 grains no effect.
    41 grains no effect.
    31 grains kill.

197. Bismarck Brown:
    246 grains produced vomiting.
    118 grains produced vomiting and general depression.
    38½ grains daily for 30 days, no effect.

398. Naphthol Green B:
    600 grains produced green urine and conjunctiva.
    292 grains produced no effect.
    128 grains produced green urine and conjunctiva.

II. Those colors that have not been observed to give negative results, but which have caused certain effects:

11. Sudan I:
    118 grains produced colored urine, vomiting, and albuminuria.

17 and 18. Chrysoidin Y and R:
    79 grains produced albuminuria.
    74 grains for 30 days produced no albuminuria, but a loss of 12½ per cent of body weight.

88. Diphenylamin Orange:
    216 grains produced albuminuria.
    128 grains produced phenol in urine and albuminuria.

138. Fast Brown G:
    237 grains produced diarrhea and loss of appetite.
    218 grains produced diarrhea.

III. *Rabbits:*

1. Picric Acid:
    24.5 grains for 90 days, no effect.
    45 grains for 19 days kill.

86. Orange II:
    292 grains, no effect.
    933 grains kill.

95. Metanil Yellow:
    216 grains, no effect.

IV. *Humans:*

1. Picric Acid:
    8.3 grains no effect.
    13.8 grains no effect.
    Invalids and children can not stand this color.

2. Dinitrocresol:
    42 grains kill.

86. Orange II:
    1½ grains, no effect.
    3 grains, headache, vertigo, dryness of throat, and poor general condition.

95. Metanil Yellow:
    1½ grains, no effect.
    3 grains, no effect.

From Classes I and II above, it would seem to appear—

(*a*) That if a dog is killed the color is harmful, even though it take as much as 603 grains, or as little as 20.4 grains per 100 pounds body weight to kill.

(*b*) That if albuminuria is produced in dogs by as little as 79 grains, or as much as 128 grains, the color is harmful.

(*c*) That if diarrhea is produced in dogs by as much as 218 grains, or as little as 50 grains, the color is harmful.

(*d*) That even if a dog can take as much as 407 grains, the color may be harmful.

From Class III it would appear—

(*a*) That even if rabbits can stand as much as 292 grains, or as little as 24.5 grains, the color is harmful.

From Class IV it would appear—

(*a*) That if 42 grains kill a human, the color is harmful.

(*b*) That even if humans can stand as much as 3 grains without untoward effect, the color is harmful.

The conclusions that may be drawn from these data are:

1. If a dog is killed by—
    (*a*) 603 grains per 100 pounds body weight, the color may be harmful.
    (*b*) 582 grains per 100 pounds body weight, the color may be harmless.
2. If a dog can bear without effect—
    (*a*) 407 grains per 100 pounds body weight, the color may be harmful.
    (*b*) 198 grains per 100 pounds body weight, the color may be harmless.
3. If albuminuria is produced in a dog by—
    (*a*) 38 grains, or 143 grains per 100 pounds, body weight the color may be harmless.
    (*b*) 79 grains, or 128 grains per 100 pounds body weight, the color may be harmful.
4. If diarrhea is produced in a dog by—
    (*a*) 70 grains per 100 pounds body weight, the color may be harmless.
    (*b*) 50 grains per 100 pounds body weight, the color may be harmful.
5. If vomiting and albuminuria are produced in a dog by 127 grains per 100 pounds body weight, the color is not necessarily harmful.
6. If small amounts of color produce in a dog diarrhea or albuminuria, and larger amounts do not, the color may be harmless.
7. Even though rabbits can withstand 292 grains per 100 pounds body weight, the color is not necessarily harmless, but may be harmful.
8. If 42 grains kill a human, the color is harmful.
9. If a human can withstand 3 grains without effect the color is not necessarily harmless, but may be harmful.
10. If a human can not withstand 3 grains, even though it can withstand 1½ grains, the color is not necessarily harmless, but may be harmful.

Lehmann (*Methoden der praktischen Hygiene, 1890, p. 545*) says:

> I regard such substances as harmful to health which when fed to a sound dog in doses of a few decigrams per day produce at once, or after a few repetitions of the dose, disturbances in the health of the dog; on the other hand, dyes which in doses of from one to several grams can be taken for weeks on end without causing any disturbance or only slight intestinal disturbances or a slight and passing albuminuria can be regarded as harmless. It should never be forgotten that a few milligrams of a coal-tar color dyes very strongly and it is not easy—even by most extraordinary use of colored objects, e. g., by children—that more than milligrams, at most centigrams, of dye can be introduced into the human stomach. In extremely large doses many substances, for example, all our condiments, are naturally harmful.

Of 65 dogs weighed and experimented on by Chlopin the average weight was 9.2 kilograms, or 20.3 pounds; almost half the dogs (30) weighed between 6 and 11 kilos.

To adapt the rules of Lehmann to a basis of grains per 100 pounds of body weight, assuming the average weight of a dog to be 20 pounds, it is only necessary to multiply the weight of color by 5 and by 15.432. Doing so, the following data are obtained:

A. Harmful colors produce bad effects in doses of a "few" decigrams, i. e., multiples of a half gram; the word "few" is not sharply defined, but the rule means a "few" times 7.7 grains.

B. Harmless colors produce, when continuously fed, no bad effect in multiples of 10 of the doses of A, above, i. e., multiples of 77 grains.

C. Slight diarrhea and slight, temporary albuminuria are not to count against the color.

Applying these rules to the preceding colors discloses that they were apparently not so used, in selecting the harmless and harmful colors in the Confectioners' List.

LEHMANN'S RULES.

The Lehmann rules as just interpreted were applied to the data just given. As a result of such application the conclusion is reached that of the 15 colors classed as harmless (foregoing classes I, II, and III), 6 would be classed as doubtful by the Lehmann rules, namely:

13. Ponceau 4 GB,
8 and 9. Fast Yellow Y and R,
55. Ponceau 2R,
65. Fast Red B,
103. Azorubin S,
105. Fast Red E,

and the remaining 9 would have been classed as harmless.

Of the 11 classed as harmful (foregoing classes I, II, III, and IV), 2 would have been regarded as harmless, namely:

398. Naphthol Green B,
138. Fast Brown G,

3 would have been classed as doubtful, namely:

197. Bismarck Brown,
11. Sudan I,
88. Diphenylamin Orange,

and the remaining 6 would have been classed as harmful.

SANTORI'S WORK AS A GUIDE TO A RULE.

Santori regards the following six dyes as harmless: 457 (188), 467 (74), 477 (129.5), 480 (156), 599 (64), 692 (467).

The first is the Green Table number and the bracketed figure the average number of grains per 100 pounds per day for 30 days.

In the case of Nos. 457 and 599 the animal was wholly normal throughout the test and the autopsy showed only normal conditions.

In the case of No. 477 the only disturbance was colored feces and the autopsy showed only normal conditions.

In the case of No. 480 the only disturbance was colored feces, but the autopsy showed a pea-green kidney.

In the case of Nos. 467 and 692 there was only vomiting; in the case of No. 467 the autopsy showed everything normal; whereas in the case of No. 692 the autopsy disclosed a swollen kidney.

Therefore, according to Santori, a dye that causes colored feces, even with colored kidney, is harmless. Also a dye that causes vomiting and swollen kidney is harmless. On the other hand, a dye (572) which produces no change or symptom observable during life, but fatty degeneration of the liver is shown at the autopsy, is a harmful dye. This lack of conformity makes the relation between dosage, symptoms, and harmfulness or harmlessness more confusing and perplexing.

YOUNG'S RULE.

It must be remembered that smaller amounts of drugs, and, therefore, of coal tar colors, effect children as a rule than are effective upon adults. Taking Young's rule as a guide, it appears that generally the effective dose for a 3-year-old child is one-fifth the effective adult dose; for a 4-year-old child one-fourth; for a 6-year-old child one-third; for an 8-year-old child two-fifths and for a 12-year-old child one-half the effective adult dose. All of this should be taken into account in drawing conclusions from experiments as to the harmlessness of any coal-tar dye upon humans. Very little attention has, however, been paid to this aspect of the matter in spite of the fact that colored foods, confectionery, pastry, beverages, and the like are partaken of by children and in many cases such articles are prepared for the sole or particular consumption of the very young.

From the foregoing data it seems clear that deductions as to the harmlessness or harmfulness of coal-tar dyes when administered to dogs, and not based upon autopsies, are not final nor conclusive as to the effect upon the dog. The extent to which such deductions are correctly transferable to humans is likewise not established.

XI. OIL-SOLUBLE OR FAT COLORS.

Oil-soluble colors are used for coloring fats, such as butter, oleomargarine, edible oils, and the like; of the coal-tar colors the oil-soluble colors are chemically nonsulphonated azo-colors.

The nonsulphonated azo-colors which have been physiologically examined are:

11. Sudan I (anilinazo-b-naphthol) (2).
16. Butter Yellow (anilin-azo-dimethylanilin).
17. Chrysoidin Y (anilin-azo-m-phenylene-diamin) (2).
18. Chrysoidin R (anilinazo-m-tolylene-diamin) (1).
41. Chrysoidin R (o-toluidin-azo-m-tolylene-diamin).
197. Bismarck Brown (m-phenylene-diamin-disazo-m-phenylene-diamin) (4).
201. Manchester Brown (m-tolylene-diamin-disazo-m-tolylene-diamin) (2).

The numbers preceding the trade names are the Green Table numbers; the scientific names appear in parentheses; and the number of dealers offering the colors on the United States market in the summer of 1907, out of a possible 12, appear after the scientific name, also in parentheses.

For all but No. 201 contradictory statements occur in the compiled literature; for No. 201 only unfavorable reports were found.

There were on the United States market in the summer of 1907 the following oil-soluble colors:

10. Sudan G (anilin-azo-resorcin) (1).
11. Sudan I (anilin-azo-b-naphthol) (2).
49. Sudan II (xylidin-azo-b-naphthol) (1).
60. Carminaph Garnet (a-naphthylamin-azo-b-naphthol) (1).

Of these No. 11 had been reported on contradictorily, and the remaining three had not been reported on at all. In addition to these the following three oil-soluble colors, not listed in the Green Tables and not reported on in literature, were wanted:

1. o-Toluidin-azo-b-naphthylamin (which does not seem to be described in literature in any way).
2. Amidoazo-toluol.
3. Anilin-azo-b-naphthylamin.

So that out of a total of seven oil-soluble colors on the United States market in the summer of 1907 only one had been examined physiologically and that with contradictory results. As before stated, these oil-soluble colors all belong to the class of nonsulphonated azo-colors.

Fraenkel (p. 575) says: "When, however, the azo-colors contain no sulpho-group (i. e., are nonsulphonated) they are poisonous. Thus for example, Bismarck Brown * * * Sudan I." Yet meta-nitrazotin, a nonsulphonated color not in the Green Tables, and probably not upon the market anywhere, and which is meta-nitranilin-azo-b-naphthol, is according to Weyl nonpoisonous.

Fraenkel (p. 575) also stated:

> The fact that the monazo colors examined by Cazeneuve and Lépine are harmless, as above stated, is equally explained by the constitution of these substances. Those two investigators examined (omitting the trade names) a-naphthylamin-sulphoacid-azo-a-naphthol-a-sulphoacid, a-naphthylamin-sulphoacid-azo-b-naphthol-disulphoacid, a-naphthylamin-azo-b-naphthol-disulphoacid, xylidin-azo-b-naphthol-disulphoacid, sulphanilicacid-azo-a-naphthol, Amido-azo-toluene-disulphonicacid.
>
> These substances are all sulphoacids and the sulpho-groups here effect the depoisoning of the original substance.

Examining this statement it therefore appears that in Fraenkel's opinion, at least, a-naphthylamin-azo-a-naphthol, a-naphthylamin-azo-b-naphthol, xylidin-azo-b-naphthol, anilin-azo-a-naphthol, and amido-azo-toluol are in and of themselves poisonous substances, and are rendered nonpoisonous by sulphonation. Diligent search through the literature has failed to uncover any original communications of investigators to that effect.

With but one known exception all the nonsulphonated azo-colors are regarded by Fraenkel as poisonous. The colors 17, 18, 41, 197, and 201 on page 159 are not used as oil-soluble colors and therefore form no comparative basis for judging oil-soluble colors except that both are nonsulphonated azo-colors; in Fraenkel's opinion, however, such a comparison is wholly justified and proper.

Excluding now 17, 18, 41, 197, and 201 for the purposes of comparison, the following tabulation is made:

Oil-soluble nonsulphonated azo-colors on the market, 1907, deemed poisonous by Fraenkel.

Oil-soluble colors.	On United States market.	Fraenkel calls poisonous.
anilin-azo-resorcin	x	....
anilin-azo-b-naphthol	x	x
xylidin-azo-b-naphthol	x	x
a-naphthylamin-azo-b-naphthol	x	x
o-toluidin-azo-b-naphthylamin	x	....
amido-azo-toluol	x	x
anilin-azo-b-naphthylamin	x	....
a-naphthylamin-azo-a-naphthol	....	x
anilin-azo-a-naphthol	....	x

That is, the only ones on the United States market concerning which no expression of specific opinion is to be found in Fraenkel are the following three: anilin-azo-resorcin, toluidin-azo-b-naphthylamin, and anilin-azo-b-naphthylamin, and nothing is published on the physiological action of any of them specifically. The probability, however, would seem to be that they are not harmless, and until their harmlessness is positively established their exclusion from the permitted list of coal-tar colors for use in foods seems to be the only safe and proper course to be followed. The physiological action of so-called "Butter Yellow" No. 16 of the Green Tables (see p. 85), merely strengthens this conclusion.

XII. RULES AND REASONS FOR SELECTING THE SEVEN COLORS PERMITTED BY F. I. D. 76.

STATEMENT OF RULES.

In view of the confusion and uncertainty disclosed in the foregoing literature relative to the physiological action of coal-tar colors, the difficulties in the way of making a list of coal-tar colors to be permitted in the coloring of food products are seen to be by no means slight. The following rules governing selection were in mind during the making of the list given in Food Inspection Decision No. 76, and the closeness with which they were followed is discussed on page 166.

Rule I. All colors which have not been physiologically tested either upon animals or man shall not be permitted for use in foods.

Rule II. All coal-tar colors which have been examined physiologically with contradictory results shall not be permitted for use in foods.

Rule III. All coal-tar colors which have been examined physiologically and have been declared to be of doubtful harmlessness shall not be permitted for use in foods.

Rule IV. Only those coal-tar colors whose chemical composition was definitely disclosed or otherwise ascertained, and which were on the United States market in the summer of 1907, and which have been examined physiologically and with no other than a favorable result shall, for the present, be permitted for use in foods.

ANALYSIS OF THREE RECOMMENDATIONS MADE TO THE DEPARTMENT OF AGRICULTURE.

These rules were formulated as a guide in view of the divergent opinions expressed in three different recommendations to the Department of Agriculture. One of these recommendations suggested that permitted colors be designated by nine titles. Comparison of these titles with the Green Tables and with the tabulated survey of the unfavorable, favorable, and contradictory literature corresponding to the Green Table numbers (p. 63) discloses the following facts:

Comparison of nine suggested color titles with corresponding Green Table numbers and the reports on the same.

Name.	Green Table No.	Unfavorable.	Favorable.	Contradictory.	No report.[1]	Name.	Green Table No.	Unfavorable.	Favorable.	Contradictory.	No report.[1]
Chrysoidin	17			x		Ponceau	147				x
	18			x			148				x
	41			x			150				x
Tropæolin	84			x			160			x	
	85		x				163			x	
	86			x			169		x		
	87			x		Bordeaux	65		x		
	88			x			107		x		
	95			x			157				x
Azoflavin	92		x				170				x
Roccellin	102		x				171				x
Ponceau	13			x			198				x
	15			x			244				x
	44				x	Biebrich Red (?)	20				x
	55			x		Sulphonated Fuchsin	462		x		
	56				x						
	57				x	Naphthol Yellow S	4		x		
	108				x						
	114				x						
	146				x	Total	36	0	8	13	15

[1] Of physiological tests in literature compiled.

Therefore, under 9 titles 36 different chemical individuals would be placed upon the permitted list, of which only 8, or less than 25 per cent, have been examined physiologically with only favorable results, and 28, or more than 75 per cent, had either not been examined at all physiologically or with contradictory results.

The 8 of these chemical individuals examined with only favorable results, and the number of sources out of a possible 12 offering them on the United States market in the summer of 1907, are as follows:

Colors reported on favorably and number of dealers handling same.

Green Table No.	Sources offering.	Green Table No.	Sources offering.
[1] *4*	10	102	0
65	2	*107*	7
85	2	169	1
92	0	462	2

[1] The italicized Green Table numbers are those of the permitted list of Food Inspection Decision No. 76.

Another recommendation suggested the permissive use of 42 entries in the Green Tables, which are tabulated below in the same manner as the suggestions in the preceding recommendation:

Comparison of 42 recommended Green Table numbers with reports in the literature.

Green table No.	Un-favor-able.	Favor-able.	Equiv-ocal.	No report.[1]	Green table No.	Un-favor-able.	Favor-able.	Equiv-ocal.	No report.[1]
4	....	x	....	....	427	....	....	x	....
8	....	....	x	....	434	x	....	....	....
17	....	....	x	....	448	....	....	x	....
50	....	....	....	x	451	....	....	x	....
53	....	....	....	x	456	....	....	....	x
54	....	....	....	x	457	....	....	x	....
55	....	....	x	....	462	....	x	....	....
65	....	x	....	....	477	....	x	....	....
85	....	x	....	....	478	x	....	....	....
86	....	....	x	....	479	x	....	....	....
89	....	x	....	....	480	....	....	x	....
92	....	x	....	....	504	....	....	x	....
94	x	....	....	....	512	....	x	....	....
102	....	x	....	....	513	....	....	....	x
103	....	x	....	....	514	....	....	....	x
107	....	x	....	....	516	x	....	....	....
138	....	....	x	....	*517*	....	x	....	....
163	....	....	x	....	518	....	....	....	x
166	....	x	....	....	562	....	....	....	x
240	....	x	....	....	601	....	....	x	....
269	....	....	x	....					
287	....	....	x	....	Total	5	14	15	8

[1] Of physiological tests in compiled literature.

Applying the conclusions hereinbefore reached to these entries, it is found that 14 out of the 42 colors recommended, or exactly one-third, had been examined physiologically with only favorable results, and the remaining two-thirds had been either examined physiologically with only unfavorable or with conflicting results, or had not been examined at all.

The 14 chemical individuals examined with favorable result and the number of sources out of a possible 12 offering them on the United States market in the summer of 1907 are as follows:

Colors reported on favorably and number of dealers handling same.

Green table No.	Sources offering.	Green table No.	Sources offering.
[1] *4*	10	*107*	7
65	2	166	0
85	2	240	1
89	1	462	2
92	0	477	0
102	0	512	3
103	6	577	5

[1] The italicized Green Table numbers are those of the permitted list of Food Inspection Decision No. 76.

The third of these recommendations suggested 27 different chemical individuals for permissive use; these are tabulated below in the same manner as the suggestions in the preceding recommendations.

Twenty-seven colors recommended for use and reports in the literature on the same.

Green table No.	Unfavorable.	Favorable.	Equivocal.	No report.[1]	Green table No.	Unfavorable.	Favorable.	Equivocal.	No report.[1]
4	....	x	....	....	481	....	....	....	x
16	....	....	x	....	512	....	x	....	....
17	....	....	x	....	516	x	....	....	....
18	....	....	x	....	517	....	x	....	....
41	....	....	x	....	518	....	....	....	x
65	....	x	....	....	521	....	x	....	....
85	....	x	....	....	532	x	....	....	....
102	....	x	....	....	584	....	....	x	....
197	....	....	x	....	601	....	....	x	....
201	x	....	....	....	602	x	....	....	....
398	....	....	x	....	650	....	....	x	....
457	....	....	x	....	*692*	....	x	....	....
462	....	x	....	....					
476	....	....	....	x	Total	4	10	10	3
477	....	x	....	....					

[1] Of physiological tests in literature compiled.

Therefore, applying the same method of drawing conclusions as in the case of the preceding two recommendations, it appears that 10 out of the 27 suggested chemical individuals had been examined physiologically with only favorable results, and the remaining 17 had either not been examined at all or with unfavorable or conflicting results. The 10 chemical individuals examined with favorable results, and the number of sources out of a possible 12 offering them on the United States market in tho summer of 1907, are as follows:

The 10 recommended colors favorably reported on in the literature and the dealers handling same.

Green Table No.	Sources handling.	Green Table No.	Sources handling.
[1] *4*	10	477	0
65	2	512	3
85	2	*517*	5
102	0	521	0
462	2	*692*	3

[1] The italicized Green Table numbers are those of the permitted list of Food Inspection Decision No. 76.

All of the colors included in these three recommendations concerning which only favorable reports were found in the literature are given in the following table:

Recommended colors favorably reported on in the literature.

Green Table No.	Recommendations.				Dealers offering.	Green Table No.	Recommendations.				Dealers offering.
	I.	II.	III.	Total.			I.	II.	III.	Total.	
[1] *4*	x	x	x	3	10	240	......	x	......	1	1
65	x	x	x	3	2	462	x	x	x	3	2
85	x	x	x	3	2	477	......	x	x	2	0
89	......	x	......	1	1	512	......	x	x	2	3
92	x	x	......	2	0	*517*	......	x	x	2	5
102	x	x	x	3	0	521	......	......	x	1	0
103	......	x	......	1	6	*692*	......	......	x	1	3
[1] *107*	x	x	......	2	7						
166	......	x	......	1	0	Total .	8	14	10	......	....
169	x	......	......	1	1						

[1] The italicized Green Table numbers are those of the permitted list of Food Inspection Decision No. 76.

This table is now rearranged to show—

I. *The recommended colors not offered in the United States market in the summer of 1907.*

Green Table No.	Recommended by—	Green Table No.	Recommended by—
92	2	477	2
102	3	521	1
166	1		

II. *The recommended colors offered on the United States market in the summer of 1907.*

Green Table No.	Recommended by—	Sources handling.	Green Table No.	Recommended by—	Sources handling.
[1] *4*	3	10	169	1	1
65	3	2	240	1	1
85	3	2	462	3	2
89	1	1	512	2	3
103	1	6	*517*	2	5
107	2	7	*692*	1	3

[1] The italicized Green Table numbers are those of the permitted list of Food Inspection Decision No. 76.

It appears that out of 17 different chemical individuals suggested by these 3 recommendations jointly, 5 were not on the United States market in the summer of 1907, and 12 were on that market; also that of these 12, 4 were suggested by all 3 recommendations; 3 were wanted by only 2 out of the 3, and the remaining 5 were spoken for by only one. Examining all of the recommendations, it appears that out of a total of 79 recommendations 17 had been examined with only favorable results, and that the remaining 62 were either not examined or had been examined with unfavorable or conflicting results.

To add to the confusion the third recommendation mentioned suggested that the following Green Table numbers should not be permitted for use in food products because they are harmful: 1; 2; 3; 6; 86; 95; 425; 451; 454; 480; 483; 484; 487; 488; 490; 571; 584; 599; 620; 624; 650. The same paper, however, recommended that Nos. 584 and 650 be permitted for use in food products; that No. 86, suggested by both the other recommendations, be prohibited; and that Nos. 95, 451, and 480, suggested by one or the other of the remaining two recommendations, be likewise prohibited for use in food products. It must be clear to everyone that these three recommendations are not of one mind as to (1) the colors which are harmless; (2) the colors which are harmful; (3) the colors which are used or are useful in food coloring; and (4) the colors which should be used in food coloring. Such a state of affairs fully justifies rules of the scope and intent of those formulated at the beginning of this section for making a selection of permissible food colors.

PROCESS OF ELIMINATION.

From the foregoing data it appears that out of 80 different chemical individuals on the food-color market of the United States in 1907, only 16 had been examined physiologically with a favorable result (see p. 64).

These 16, arranged according to the shade produced, are as follows (numbers preceding the names are the Green Table numbers; the numbers in parentheses show the number of sources desiring the color; the italicized Green Table numbers are the ones finally selected by Food Inspection Decision No. 76):

REDS.

65. Fast Red B (Alphanaphthylamin azo-R-salt) (2).
103. Azorubin S (Naphthionic acid azo NW acid) (6).
105. Fast Red E (Naphthionic acid azo-Schaeffer acid) (1).
107. Amaranth (Naphthionic acid azo-R-salt) (7).
169. Crocein Scarlet 7B (Amidoazotolutol-monosulphonic-acid-azo-betanaphthol-monosulphonic acid B) (1).
240. Congo Red (Benzidin disazo-naphthionic acid) (1).
462. Acid Magenta (Magenta trisulphonic acid) (2).
512. Eosin A (Tetra-bromo-fluorescein) (3).
517. Erythrosin (Tetra-iodo-fluorescein) (5).
520. Rose Bengal (Dichlor-tetraiodo-fluorescein) (2).

ORANGE.

85. Orange I (Sulphanilic acid azo-alpha-naphthol) (2).

YELLOWS.

4. Naphthol Yellow (Dinitroalphanaphtholsulpho acid) (10).
89. Brilliant Yellow S (Sulphanilic acid azo-diphenylamin, sulphonated) (1).

GREENS.

433. Guinea Green B (Benzyldehyde + benzylethyanilin sulpho-acid) (1).
435. Light Green S F yellowish (Benzyldehyde+benzylethylanilin+sulphonation) (4).

BLUE.

692. Indigo Carmine (Indigo disulpho acid) (3).

Considering now the reds, and particularly 107, which, as the number appearing in parentheses after the scientific name indicates, was wanted by 7 sources out of the 12 drawn on, this being the most desired of all the reds, it would seem reasonable to believe that all the wants supplied by 65, 103, 105, 169, 240, and 462 would be covered by 107. Chemically 107 is closely allied to 65, 103, 105, and tinctorially it is likewise closely allied to 169, 240, and 462.

Nos. 512, 517, and 520 are chemically quite different from the other members of this group, and tinctorially they differ, being of a particularly brilliant shade, and tinctorially more powerful; 517 being desired by 5 out of the 12 sources drawn upon, and thus being the most desired of these three colors, was selected in the expectation that any work 512 and 520 could do in food products would be equally well done by 517. For reds, therefore, the choice fell upon 107 and 517.

Orange.—Only one color was wanted, and that by 2 out of the 12 sources, and this was placed in the permitted list.

Yellows.—The choice of No. 4, which was wanted by 10 out of the 12 sources, was made in the expectation that every purpose that 89 could serve in food products could be served also by 4.

Greens.—Tinctorially and chemically the two greens are very closely allied, and in view of the reasonableness of the expectation that 435 could do all the work of 433 as well, its choice was regarded as justified, especially as 435 was wanted by 4 out of the 12 sources, as against only one for 433.

Blue.—No. 692 being regarded as harmless by all, and being the only blue in the list, it was selected.

In this manner six out of the seven permitted colors were selected.

REASONS FOR ADDING PONCEAU 3R.

An examination of the table on page 20 discloses the fact that among the reds desired four were azo-reds made from anilin derivatives as the first component, namely:

53. Xylidin-azo-alphanaphtholdisulpho acid.
54. Xylidin-azo-Schaeffer acid.
55. Xylidin-azo-R-salt.
56. Cumidin azo-R-salt.

Each of these was wanted by 1 source out of the possible 12. This was construed as an expression of a real need in the art of food coloring for a color of this class. The reason for such a need was not then, nor is it now, apparent, but the propriety of giving even a seeming need due consideration was regarded as justified. For only one of the four desired, namely, 55, could any specific references in the literature be found, and, in fact, four references were found favorable thereto and three unfavorable thereto; thus eliminating 55 from consideration, leaving 53, 54, and 56.

The Austrian law of January 22, 1896 (see Lieber, *p. 15*), permits the use of azo colors derived from higher homologues of anilins, beginning with xylidin and sulphonated betanaphthols. As Nos. 53 and 54 were both derived from xylidin, it was regarded as safe to assume that they would probably be as objectionable as 55. This left 56 only to be considered, which color is derived from cumidin and R-salt. The cumidin portion satisfies the Austrian law and also satisfies the general law laid down in Fraenkel (*p. 162*), namely, that the greater the number of ring-methyls the less the toxic property of the resulting compound, because of the oxidation of these methyls to carboxyls in the animal system; the R-salt portion satisfies the general law laid down in Fraenkel, that the more highly sulphonated the less toxic a substance becomes, and therefore 56 is in the first portion of its composition of such a nature as to be less objectionable than 55, if 55 be objectionable.

This combination of facts, namely, the desirability of an anilin azo-red, the provision for diminishing the toxicity of 55 by the substitution of cumidin for xylidin therein, and the general provision in the Austrian food law, made it appear desirable and safe to take into account an anilin azo-red, and therefore to select 56 as probably the least objectionable, if it be at all objectionable, of the anilin azo-reds.

The full list of permitted colors was therefore extended to 7, as follows:

Red shades.—107. Amaranth; 56. Ponceau 3R; 517. Erythrosin.
Orange shade.—85. Orange I.
Yellow shade.—4. Naphthol Yellow S.
Green shade.—435. Light Green S F yellowish.
Blue shade.—692. Indigo disulpho acid.

It should be noted that with respect to tetra-iodo-fluorescein, 517 of the Green Tables, no specific investigation, pronouncing it harmless or harmful, is described in the literature. No. 516, the diodo-fluorescein, is reported specifically adversely in the literature, especially by Chlopin; the Confectioners' List, the laws of Austria, the laws of France, the rather superficial examination of No. 517 by Grandhomme, and the statement in Fraenkel (*p. 574*), that it produces no disturbance, are all taken to apply to 517 and not to 516. The identification of the color under examination with 517 has not in all cases been satisfactorily exclusive, but it is believed that the differentiation of the harmful 516 by Chlopin makes all the other references cited pertinent to 517.

With respect to 435, it must be pointed out that 434, which is the methyl instead of the ethyl derivative, has only been regarded as suspicious, and one examination of 435, namely, that of Lieber (*p. 144*), does not appear to disclose anything which would positively exclude 435.

QUALITY, CLEANLINESS, AND EFFICIENCY.

The justification for limiting the permitted colors to 6 out of 16, against which nothing unfavorable is contained in the literature and regarding which favorable statements are at hand, and the addition to these 6 of the seventh color is to be found in the very great variation in tinctorial quality, in percentage of coloring matter, in amounts of insoluble matter, both organic and inorganic, and in the amounts of organic matter not coloring matter, as well as in the large number of samples containing an amount of arsenic in excess of that permitted by the United States Pharmacopœia for the only coal-tar color therein mentioned, namely, Methylene Blue, and the varying amounts of heavy metals, such as copper, lead, and iron, mostly in excess of the limits permitted in the Pharmacopœia for various medicinal chemicals. This wide variation in quality and degree of cleanliness, all pointing to a very great difference in the care with which coal-tar colors offered for food purposes are prepared, render the conclusion safe that some control over food colors in respect to quality is desirable, necessary, and essential.

The efficiency of the 7 colors, 6 of which were selected from among the 16 considered for the purpose of making this list of permitted colors as being reasonably sure to be harmless, is evidenced by the fact that, although the addition of colors has been sought by persons interested in the food-coloring art, not one of the remaining 10 colors of those 16 has been so requested. In other words, the colors that the department has been requested to add to the permitted list were outside of the 16 colors which were on the markets of the United States in 1907, and were described in the literature in such a manner as to lead to the conclusion that they were probably not harmful.

XIII. LISTS OF COLORS SUBSEQUENTLY RECOMMENDED BY INDIVIDUALS AND ASSOCIATIONS.

Since Food Inspection Decisions Nos. 76 and 77 were published recommendations of lists of permitted colors have been made by other individuals and by a voluntary association. For the purpose of comparing these proposed lists of permissible colors with the permitted list of Food Inspection Decision No. 76, the former are now to be examined in the same manner as the coal-tar colors, on the United States market in the summer of 1907 for food coloring purposes, were examined in order to establish the permitted list of 7 colors. These recommendations cover the following lists:

1. W. Ernst's list.
2. Muttelet's interpretation of the French law.
3. Second International White Cross Congress list.
4. Beythien and Hempel's list.
5. An American manufacturer's list.
6. Béhal's list.

ERNST.

W. Ernst (*Färber Zeitung 1908, vol. 19, p. 381;* abst. *Chem. Ztg. "Reportorium" 1909, p. 89*) recommends for use in foods the following 38 titles of coal-tar colors. The Green Table numbers appear in parentheses after each title where corresponding numbers could be ascertained; the italicized numbers are those of the permitted list of Food Inspection Decision No. 76.

1. Auramin (425, 426).
2. Naphthol Yellow S (*4*).
3. Quinolin Yellow (666, 667).
4. Tartrazin (94).
5. Acid Yellow (8, 88, 95, *4*).
6. Spirit Yellow (Amidoazobenzol) (7).
7. Curcumin S (399).
8. Eosin (512, 514, 515, *517*, 521).
9. Erythrosin (516, *517*).
10. Fluorescein (510).
11. Orange II (86).
12. Crocein Orange (13).
13. Basic Oranges (?).
14. Fast Red (63, 65, 102, 103, 105, *107*, 144).
15. Amara Red (?).
16. Naphthol Red (?).
17. Azo red (62).
18. Bordeaux (65, *107*, 157, 170, 171, 198, 244).
19. Victoria Rubin (?).
20. Ponceau (13, 15, 44, 55, *56*, 57, 108, 114, 146, 147, 148, 150, 160, 163, 169, 448).
21. Rhodamin (496, 497, 498, 502, 504, 505).
22. Acid Magenta (462).
23. Safranin (583, 584, 585).
24. The Croceins (13, 104, 106, 145, 151, 160, 164, 169).
25. Acid Green (434, *435*).
26. Brilliant Fulling Green (?).
27. Malachite Green (427, 428).
28. Brilliant Green (428).
29. Water Blue (480).
30. Patent Blue (440, 442).
31. Brilliant Fulling Blue (?).
32. Domingo Blue B extra (?).
33. Methyl Violet (451, 454).
34. Acid Violet (464, 465, 467, 468, 470, 471, 472, 474, 507).
35. Bismarck Brown (197, 201).
36. Acid Brown (133, 138).
37. Nigrosin (600,602).
38. Several Acid Blacks (184 and ?).

Of these 38 titles only 11 refer to a single entry each in the Green Tables (namely 2, 4, 6, 7, 10, 11, 12, 17, 22, 28, 29); 7 titles can not be definitely connected with any entry in the Green Tables (titles 13 15, 16, 19, 26, 31, 32); one title is broader than the corresponding color in the Green Tables (title 38) and the remaining 19 titles each and all refer to more than one entry in the Green Tables.

These 31 titles embrace 88 different entries in the Green Tables; some of these entries are included in two or more titles as follows:

Green Table number.	Titles.
4	2, 5
13	12, 20, 24
65	14, 18
107	14, 18
160	20, 24
169	20, 24
428	27, 28
517	8, 9

These 88 different Green Table entries can be divided as follows on the basis of the compilation of literature on physiological action hereinbefore given (see p. 63):

Unfavorable only.—94, 164, 201, 425, 434, 502, 516, 602, 667. Total 9, or 10.2 per cent.

Favorable only.—*4*, 65, 102, 103, 105, *107*, 169, 399, *435*, 462, 467, 512, *517*, 521, 600. Total 15, or 17.0 per cent.

Conflicting.—8, 13, 15, 55, 86, 88, 95, 106, 138, 160, 163, 197, 427, 428, 448, 451, 480, 504, 584. Total 19, or 21.6 per cent.

Not reported on.—7, 44, 56 [1], 57, 62, 63, 104, 108, 114, 133, 144, 145, 146, 147, 148, 150, 151, 157, 170, 171, 184, 198, 244, 426, 440, 442, 454, 464, 465, 468, 470, 471, 472, 474, 496, 497, 498, 505, 507, 510, 514, 515, 583, 585, 666. Total 45, or 51 per cent.

According to this mode of judging only 15, or 17 per cent, of the colors suggested by Ernst for food coloring would be regarded as proper for use in foods.

These 15 embrace 4 of the 7 permitted colors of Food Inspection Decision No. 76, namely, 4, 107, 435, and 517, leaving 11 to be considered. Of these, 5 were not on the United States market in the summer of 1907, namely, 102, 399, 467, 521, and 600.

The remaining 6 are as follows (the numbers in parentheses being the number of sources out of a possible 12 offering them on the United States market in the summer of 1907): 65 (2); 103 (6); 105 (1); 169 (1); 462 (2); and 512 (3).

The reasons for the noninclusion of these in the permitted list of Food Inspection Decision No. 76 have been given on page 167.

MUTTELET'S INTERPRETATION OF THE FRENCH LAW.

Muttelet (*Annales des Falsifications, 1909, pp. 26–38*), places the following interpretation on the French regulations of December 29, 1890, and of August 4, 1908, classifying them as—

I. Those colors which are certainly permitted by those regulations.
II. Those colors whose permitted or prohibited use is doubtful.
III. Those colors which are certainly prohibited.

The Green Table numbers contained in each class are classified as follows (the italicized numbers being those of the permitted list of Food Inspection Decision No. 76): (*a*) Unfavorably reported; (*b*) favorably reported; (*c*) conflictingly reported; (*d*) not reported in the literature as to their physiological action (see p. 63); in parentheses is given the number of sources out of a possible 12 offering those colors on the United States market in the summer of 1907.

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[1] See page 167 for special reasons for including Ponceau 3R in permitted list.

I. Those colors which are certainly permitted:

(*a*) None. (*b*) *4* (10); 5 (0); 462 (2); 512 (3); *517* (5); 520 (2); 600 (0); total, 7. (*c*) 55 (2); 427 (2); 451 (5); 457 (0); total, 4. (*d*) 158 (0); 518 (2); total, 2.

II. Those colors whose permitted or prohibited use is doubtful:

(*a*) None. (*b*) 65 (2); *107* (7); 599 (0); total, 3. (*c*) 8 (5); 84 (2); 427 (2); 457 (0); 601 (1); total, 4. (*d*) 452 (2); 456 (0); 513 (0); 514 (0); total, 4.

III. Those colors which are certainly prohibited:

(*a*) 3 (0); (*b*) none; (*c*) 427 (2); (*d*) none.

It will be noted that 427 appears in all three of Muttelet's classifications. The reason for this is that 427 is or has been marketed in at least three different forms; of these the straight chlorhydrate is permitted, the oxalate and the zinc chlorid double salt are of doubtful admissibility, and the picrate is undoubtedly forbidden.

Also 457 appears in Muttelet's Classes I and II because the "Bleu Lumière" of Muttelet's Class I is indistinguishable from his "Bleu Lumière" of Class II, when using the Green Tables as a guide. Further, this list of Muttelet contains only 3 out of the 7 colors permitted in Food Inspection Decision No. 76, namely, 4 and 517 of his Class I and 107 of his Class II.

An inspection of Muttelet's Class I discloses 13 Green Table entries, of which only 7 have been reported in the literature, as herein compiled and rated, in a favorable manner; of these 7, 2 were not on the United States market in the summer of 1907 and of the remaining 5, 2 are in the permitted list of Food Inspection Decision No. 76; the remaining 3 are: 462. Acid Magenta (2); 512. Eosin (3); 520. Rose Bengal (2), and the reasons for whose noninclusion in the permitted list of Food Inspection Decision No. 76 have been given (p. 167).

SECOND INTERNATIONAL WHITE CROSS CONGRESS.

The Second International White Cross Congress, held in Paris, October 18 to 24, 1909, according to the Chemiker Zeitung, 1909, page 1227, adopted the following list of colors which were said to be proper for use in coloring food products. The figures in parentheses are the Green Table numbers; the italicized numbers are those of the permitted list of Food Inspection Decision No. 76.

1. Erythrosin (516, *517*).
2. Rhodamin B (504).
3. Bordeaux S (*107*).
4. Bordeaux G (170).
5. Fast Red E (105).
6. New Coccin (106).
7. Ponceau 2R (55).
8. Xylidin Scarlet (55).

9. Magenta (448).
10. Acid Magenta (462).
11. Orange I (*85*).
12. Naphthol Yellow S (*4*).
13. Chrysoin (84).
14. Auramin O (425).
15. Acid Green (434, *435*).
16. Lyons Blue (457).
17. Patent Blue (440, 442).
18. Paris Violet (451).
19. Acid Violet (464, 465, 467, 468, 470, 471, 472, 474, 507).
20. Black Indulins (599).
21. Sulphonated Nigrosin (602).

It will be observed that titles 7 and 8 refer to the same Green Table number; this leaves, therefore, only 20 titles to consider. These refer to 31 different Green Table numbers which are classified as (*a*) only unfavorable reports, (*b*) only favorable reports, (*c*) conflicting reports, and (*d*) no reports, in the literature hereinbefore compiled and rated (see p. 63):

(*a*) 425, 434, 516, 602; total, 4.
(*b*) *4*, *85*, 105, *107*, *435*, 462, 467, *517*, 599; total, 9.
(*c*) 55, 84, 106, 448, 451, 457, 504; total, 7.
(*d*) 170, 440, 442, 464, 465, 468, 470, 471, 472, 474, 507; total, 11.

The numbers of section (*b*), of which only favorable reports are recorded, are the only ones here of interest; they are 9 in number, or less than 30 per cent of all those included in this list, and of these 9, 5 are on the permitted list of Food Inspection Decision No. 76. The remaining 4 are the following, the number in parentheses representing the number of sources out of a possible 12 offering them on the United States market in the summer of 1907:

105. Fast Red E (1).
462. Acid Magenta (2).
467. Acid Violet 6 B, not offered.
599. Printing Blue, not offered.

The reasons for the noninclusion of these colors in the permitted list of Food Inspection Decision No. 76 have been given on page 167.

BEYTHIEN AND HEMPEL.

Beythien and Hempel (*Färber Ztg., 1909, v. 15, pp. 301, 348, 392, 436;* abst. *Chem. Ztg., 1910, p. 58*) recommend the following colors for use in food products. (The numbers in parentheses are the corresponding Green Table numbers, where such connection could be established; the italicized numbers are those of the permitted list of Food Inspection Decision No. 76.)

1. Alizarinblue (562, 563).
2. Amaranth (*107*).
3. Bordeaux Red (?).
4. Brilliant Blue (?).
5. Diamondfuchsin (448).
6. Fast Blue (477, 599, 601, 639, 640).
7. Fast Yellow R (9).
8. Fast Red (63, 65, 102, 103, 105, *107*, 144).
9. Eosin (512, 514, 515, *517*, 521).
10. Erythrosin (516, *517*).
11. Fuchsin S (462).
12. Light Green S F yellowish (*435*).
13. Indigo disulphoacid (*692*).
14. Indulins (599, 601, 603).
15. Light Blue (?).
16. Malachite Green (427, 428).
17. Methyl Violet (451, 454).

18. Naphthol Yellow S (*4*).
19. Orange I (*85*).
20. Orange L (54).
21. Paris Violet (451).
22. Phloxin (518, 521).
23. Ponceau 3R (*56*, 57).
24. Ponceau Red (?).
25. Primrose (3, 513, 514).
26. Roccellin (102).
27. Roscellin (?).
28. Rubin (448).
29. Acid Yellow S (*4*).
30. Acid Magenta (462).
31. Solid Blue (?).
32. Tropaeolin OOO (*85*, 86).
33. Waterblue (480).

For six titles (3, 4, 15, 24, 27, and 31) no corresponding Green Table number could be determined.

Of the remaining 27 titles, No. 9 includes part of No. 10, 32 includes 19, 9 includes part of 25, 8 includes 26, 17 includes 21, 11 is identical with 30, and 18 is identical with 29, all on the assumption that the correct connections between title and Green Table numbers have been made. Therefore these 27 titles are, in fact, only 25 titles; of these 25 titles, 13 refer to but one Green Table number each; the remaining 12 titles each refer to two or more Green Table numbers.

The Green Table numbers above given are now arranged in the following four classes according to the literature hereinbefore compiled and rated (see p. 63):

(*a*) Only unfavorable reports: 3, 516, 639; total, 3.

(*b*) Only favorable reports: *4*, 65, *85*, 102, 103, 105, *107*, *435*, 462, 477, 512, *517*, 521, 599, *692;* total, 15.

(*c*) Conflicting reports: 9, 86, 427, 428, 448, 451, 480, 563, 601; total, 9.

(*d*) No reports: 54, *56*,[1] 57, 63, 144, 454, 513, 514, 515, 518, 562, 603, 640; total, 13.

The 15 Green Table numbers of class (*b*) are the only ones here of interest; they include 6 out of the 7 permitted colors of Food Inspection Decision 76; of the remaining 9, 4 (102, 477, 521, 599) were not on the United States market in the summer of 1907. The remaining 5 are as follows. (The number in parentheses is the number of sources out of a possible 12 offering them on the United States market in the summer of 1907):

65. Fast Red B (2).
103. Azorubin S (6).
105. Fast Red E (1).
462. Acid Magenta (2).
512. Eosin (3).

The reasons for the noninclusion of these colors in the permitted list of Food Inspection Decision No. 76 have been given on page 167.

SUMMARY OF THREE PRECEDING RECOMMENDATIONS.

The recommendations made by Ernst, the White Cross Congress, and Beythien and Hempel are summarized in the following table:

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[1] See page 167 for special reasons for permitting use of Ponceau 3R.

Summary of recommendations from three sources.

Green Table No. (11).	Unfavorable reports only.			Number recommending.	Dealers offering.
	Ernst.	White Cross.	Beythien and Hempel.		
3	....	....	x	1	0
94	x	....	....	1	6
164	x	....	....	1	0
201	x	....	....	1	2
425	x	x	....	2	3
434	x	x	....	2	1
502	x	....	....	1	2
516	x	x	x	3	1
602	x	x	....	2	0
639	....	....	x	1	0
667	x	....	....	1	1
Total..	9	4	3	....	....

Green Table No. (19).	Favorable reports only.			Number recommending.	Dealers offering.
	Ernst.	White Cross.	Beythien and Hempel.		
4	x	x	x	3	10
65	x	....	x	2	2
85	....	x	x	2	2
102	x	....	x	2	0
103	x	....	x	2	6
105	x	x	x	3	1
107	x	x	x	3	7
169	x	....	....	1	1
399	x	....	....	1	0
435	x	x	x	3	4
462	x	x	x	3	2
467	x	x	....	2	0
477	....	....	x	1	0
512	x	....	x	2	3
517	x	x	x	3	5
521	x	....	x	2	0
599	....	x	x	2	0
600	x	....	....	1	0
698	....	....	x	1	3
Total..	15	9	15	....	....

Green Table No. (24).	Conflicting reports.			Number recommending.	Dealers offering.
	Ernst.	White Cross.	Beythien and Hempel.		
8	x	....	....	1	5
9	....	....	x	1	1
13	x	....	....	1	6
15	x	....	....	1	0
55	x	x	....	2	2
84	....	x	....	1	2
86	x	....	x	2	8
88	x	....	....	1	0
95	x	....	....	1	2
106	x	x	....	2	5
138	x	....	....	1	0
160	x	....	....	1	0
163	x	....	....	1	0
197	x	....	....	1	4
427	x	....	x	2	2
428	x	....	x	2	3
448	x	x	x	3	4
451	x	x	x	3	5
457	....	x	....	1	0
480	x	....	x	2	1
504	x	x	....	2	5
563	....	....	x	1	0
584	x	....	....	1	1
601	....	....	x	1	1
Total..	19	7	9	....	....

Summary of recommendations from three sources—Continued.

Green Table No. (51).	Not reported on.			Number recommending.	Dealers offering.
	Ernst.	White Cross.	Beythien and Hempel.		
7	x	....	....	1	0
44	x	....	....	1	0
54	....	....	x	1	1
56	x	....	x	2	1
57	x	....	x	2	0
62	x	....	....	1	0
63	x	....	x	2	0
104	x	....	....	1	1
108	x	....	....	1	2
114	x	....	....	1	0
133	x	....	....	1	0
144	x	....	x	2	0
145	x	....	....	1	0
146	x	....	....	1	2
147	x	....	....	1	0
148	x	....	....	1	0
150	x	....	....	1	0
151	x	....	....	1	0
157	x	....	....	1	0
170	x	x	....	2	0
171	x	....	....	1	0
184	x	....	....	1	0
198	x	....	....	1	0
244	x	....	....	1	0
426	x	....	....	1	0
440	x	x	....	2	1
442	x	x	....	2	0
454	x	....	x	1	0
464	x	x	....	2	1
465	x	x	....	2	0
468	x	x	....	2	1
470	x	x	....	2	0
471	x	x	....	2	0
472	x	x	....	2	0
474	x	x	....	2	0
496	x	....	....	1	0
497	x	....	....	1	0
498	x	....	....	1	0
505	x	....	....	1	0
507	x	x	....	2	0
510	x	....	....	1	4
513	....	....	x	1	0
514	x	....	x	2	0
515	x	....	x	2	0
518	....	....	x	1	2
592	....	....	x	1	0
583	x	....	....	1	0
586	x	....	....	1	0
603	....	....	x	1	0
640	....	....	x	1	0
666	x	....	....	1	0
Total	45	11	13	....	....

These three recommendations embrace 105 different Green Table numbers. The following table discloses the conformity of the composite of these three recommendations to the United States market in the summer of 1907:

Number of suggested samples in the three supplementary lists found on the market, 1907.

Data.	Total in class.	On United States market.
Unfavorable	11	7
Favorable	19	13
Conflicting	24	17
Not reported	51	10
Total	105	47

UNPUBLISHED RECOMMENDATIONS OF A MANUFACTURER.

In addition to these published recommended lists the following recommendation has been made by letter by a manufacturer who contributed specimens of food colors to the United States market in the summer of 1907:

That for the following permitted colors of Food Inspection Decision No. 76, there be substituted certain colors, the Green Table numbers alone being here given:

Permitted.	Substitute.
4	94
56	53
85	15
517	521

In regard to these proposed substitutes it is to be said that Nos. 15 and 521 were not on the United States market in the summer of 1907, according to the canvass made and described in Section I, Nos. 53 and 94 were on that market, No. 53 had not been examined physiologically, and No. 94 had been examined physiologically with only unfavorable results. The way was, therefore, not open to placing any of these colors on the permitted list under the procedure adopted. However, had No. 15 been on the market it would not have been placed on the permitted list, because it has been examined physiologically with contradictory results, while No. 521 might have been placed on the permitted list because it seems to have been examined physiologically and with only favorable results.

BÉHAL.

As the result of careful investigation Béhal (*Revue Générale des Matières Colorantes, 1910, p. 131*) suggests the use of 21 definite chemical individuals which are given in the following. With the tabulation on page 63 as a guide these 21 colors are classified as unfavorable, favorable, contradictory, and not reported on. The italicized figures are in the permitted list of Food Inspection Decision No. 76.

UNFAVORABLE (1).

425. Auramin O (3).

FAVORABLE (11).

4. Naphthol Yellow S (10).
65. Bordeaux B (2).
85. Orange I (2).
105. Fast Red (1).
107. Amaranth (7).
435. Light Green S F yellowish (4).
462. Acid Magenta (2).
467. Acid Violet 6B (none).
512. Eosin (2).
517. Erythrosin (5).
520. Rose Bengal (2).

CONTRADICTORY (6).

55. Ponceau 2 R (2).
84. Chrysoin (2).
106. New Coccin (5).
427. Malachite Green (2).
451. Methyl Violet (5).
480. Water Blue (1).

NOT REPORTED ON (3).

54. Scarlet R (1).
64. Crystal Ponceau (1).
440. Patent Blue (1).

The five in the "favorable" list which were on the United States market in the summer of 1907, but are not in the permitted list of Food Inspection Decision No. 76, namely:

65. Bordeaux B (2);
105. Fast Red (1);
462. Acid Magenta (2);
512. Eosin (2);
520. Rose Bengal (2),

are tinctorially provided for in that list; only one of the 21 colors recommended by Béhal was not on the market of the United States in the summer of 1907, namely, 467 Acid Violet 6B, and this is in the "favorable" list. The reasons for the non-inclusion of these colors are given on page 167.

CONCLUSIONS.

It is clear from an inspection of the preceding analysis of the six lists just given that the confusion referred to on page 165 as existing in the three recommendations made before the formulation of the permitted list of Food Inspection Decision 76 is not at all diminished by these six lists published after the announcement of that decision, as is shown in the following table:

Analysis of six lists published after the issuance of F. I. D. 76.

List of—	Total of dyes wanted.	Reports on physiological effect are—			Physiological effect not reported.	Number of dyes wanted and contained in F. I. D. 76.
		Unfavorable.	Favorable.	Contradictory.		
Ernst	88	9	15	19	45	4
Muttelet's class I	7	....	7	....	....	2
White Cross	31	4	9	7	11	5
Beythien and Hempel	40	3	15	9	13	6
Manufacturer	4	1	1	1	1	0
Béhal	21	1	11	6	3	5

The same wide differences of opinion as to the four points discussed on page 166 not only continue but are accentuated.

XIV. CHEMICAL EXAMINATION OF THE SEVEN PERMITTED COLORS, 1907.

NEED OF CHEMICAL CONTROL.

The most striking thing, from the chemist's point of view, in the literature relative to the physiological action of coal-tar colors, is the almost universal absence of the results of chemical examination or identification of the materials subjected to physiological test. There is hardly any description or statement of the strength or the concentration of the materials examined, or of the amount or nature of materials not coloring matter present in the substances subjected to these physiological tests. It is true that some publications give identification tests, but few assert the identity of the material subjected to certain physiological tests, with the description so given.

The need for some such chemical control as to identity and quality must be apparent to all having experience with the commercial varieties or grades of coal-tar colors. Many of these commercial brands of coal-tar colors contain added coloring matter other than the principal constituent for the purpose of correcting some defect unavoidably arising during the manufacture. Where those commercial brands are sold for textile and other manufacturing purposes there is no valid objection to such practice, because in such uses the tinctorial properties and effects of the commercial brands are the things the buyers desire and pay for, and the exact nature of the materials accomplishing the results is in reality of secondary importance; but when coal-tar colors are to be used in foods, and in addition to tinctorial effect the absence of any physiological action is necessary, it is at least of doubtful propriety to market such corrected or adjusted brands unless the correction or the adjustment be made with harmless colors.

There are a few instances in the relevant literature where it was attempted to explain the different results attained by observers by the difference in the composition of the materials subjected to physiological test, and there are other instances showing the difficulties encountered in obtaining specimens of coal-tar colors which would always give favorable physiological result.

The variation in composition of coal-tar colors may be exemplified by the following excerpts from the literature:

1. Weyl (*p. 91*), discussing the differences between his results and those of Cazeneuve and Lépine with Naphthol Yellow S, states as follows:

> It is to be noticed, however, that Cazeneuve and Lépine state that the Naphthol Yellow used (Jaune NS) was difficultly soluble, so that they probably experimented

> with another substance. My own investigations were made on dogs with a preparation for which I am indebted to the kindness of Dr. G. Schultz, of the Aniline Manufacturing Co. of Berlin. It was purified by precipitation and recrystallization.

2. Fraenkel (*p. 578*) expresses the opinion that the harmful results observed with Metanil Yellow (95 of the Green Tables) may be due to diphenylamin contained in the color, which diphenylamin may be a decomposition product of the Metanil Yellow, particularly since, as Weyl states (*p. 130*), it "smelled strongly of diphenylamin."

3. Pfeffer (*Unterschungen aus dem Botanischen Institut zu Tuebingen, Vol. II, p. 186*) says:

> In repeating my experiments I beg that it be considered that differences in observation may be caused by the quality of the coloring matters. For quite apart from the fact that the coloring matters are in part variable mixtures of various compounds, and not infrequently contain foreign admixtures, the quality of the goods brought into market may change from time to time. Thus formerly magenta was the acetate, whereas to-day it is the hydrochlorid. Also foreign admixtures may be poisonous or may exert no influence on the absorption of color.

4. Stilling (*Archiv. Exper. Path. Pharm., vol. 28, 1891, p. 352*) says:

> With respect to the blue pyoctanin, I have during the entire time been engaged in ascertaining the most effective. The things marketed by E. Merck as P. cœruleum have therefore now become uniform and homogeneous, which could not be the case at the beginning. The P. cœruleum now furnished by E. Merck is the hydrochlorid of pure hexamethylpararosanilin.

The variable degrees of purity attained by products of this kind on the market was apparently the first cause of the prohibition by the German Government of the coal-tar color known as Corallin for use in food products—not because Corallin itself was harmful, but because in the manufacture of this product it was so apt to retain impurities which in and of themselves produced bad effects, so that this particular color was specifically excluded by the German Government.

In a few cases it is stated that the substance subjected to physiological test had been purified, but these descriptions are hardly such as to enable others to arrive at substantially the same result with reasonable certainty.

5. Stilling in his monograph entitled Anilin Farbstoffe als Antiseptica (Strassburg, 1890, Pt. I, p. 16) says:

> The foregoing (relative to the action of Methyl Violet on the eyes of rabbits) holds only for pure substances. Many anilin colors of otherwise very antiseptic properties are contaminated with arsenic, particularly the otherwise useful Ethyl Violet. With such substance serious poisonings and death can be produced in experimental animals.

6. Stilling in Part II of the same monograph, pages 5 and 6, says:

> Such substances (coal-tar colors) must be chemically pure and can not, for example, be any mixture of various blue or violet dyes. There is therefore a great difference whether a substance be tested only bacteriologically or also in addition physiologically and therapeutically. Two preparations may be antiseptically wholly equal, but the one preparation may contain harmful admixtures which produce violent irritations,

7. Thus Weyl, speaking of his Metanitrazotin (*p. 120*), describes the purification method as follows:

> It is dissolved in warm alcoholic solution of sodium hydroxid filtered and precipitated with hydrochloric acid. The precipitate is freed from the adhering liquid by the aspirator and washed with hot water.

8. Weyl says of his Metanil Yellow (*p. 130*):

> For purification the color was dissolved in water filtered and separated by the addition of sodium acetate. The yellow mass was freed from the adherent liquid by the filter pump and dissolved in hot alcohol, in which it is difficultly soluble, and obtained from this in the form of yellow crystals. The material used for the experiment was almost pure, as the following analytical statement shows: 0.4895 gram of the color dried in 105° gave 0.084 sodium sulphate. Sodium required, 6.1; found, 5.6. (Note.—This amounts to 91.8 per cent of theory.)

9. Weyl (*Zts. Hygiene, 1889, Vol. II, p. 34,* On Safranin Poisoning) describes the difficulties he had in obtaining safranin on the market that was clean or pure; all preparations were free from arsenic, and contained small amounts of iron, chlorin, and traces of chromium. In one specimen the ash amounted to 4.8 per cent. The theoretical percentage of nitrogen in pure safranin is, according to Weyl, 15.3 per cent; in two commercial products he found 12.7 and 12.3 per cent, respectively (83 and 80.4 per cent of theory); he recrystallized the specimen containing 12.7 per cent twice from dilute hydrochloric acid; the first recrystallization produced a specimen containing 13.8 per cent (90.2 per cent of theory) of nitrogen; the second recrystallization produced a substance containing 14 per cent (91.5 per cent of theory) nitrogen.

10. Chlopin in his book (*p. 110*) says:

> Nevertheless, each dye was tested by me personally with the usual reagents and the dyeing of fabric in order to avoid the confounding of one dye with another. I convinced myself, from my experience, that not only druggists but the home offices of the makers occasionally send dyes which do not correspond to the requirements and resemble them only in name.
>
> (*Page 114.*) All the dyes, which according to my experiments proved to be poisonous, were carefully examined for contents of arsenic, chromium, and injurious metals, and were found to be free from these admixtures.

FIRST METHODS OF ANALYSIS USED.

In the summer of 1907 there were on the United States market 30 different specimens of the seven permitted colors of Food Inspection Decision No. 76. A chemical examination of those 30 specimens would disclose the qualitative conditions of the market so far as these seven permitted colors were concerned, and it was expected that certain limits for standards of cleanliness would be fixed by such an examination.

In examining these 30 specimens of the seven permitted colors chemically the following determinations were made:

1. Moisture.
2. Chlorin as chlorids.
3. Sulphated ash, together with its iron, aluminum, calcium, and copper content, and the determination of the sulphuric acid in the sulphated ash.
4. Total sulphur.
5. Gutzeit test (test 17 of the United States Pharmacopœia, eighth revision).
6. Heavy metals test (test 121 of the United States Pharmacopœia).
7. Total insolubles, together with the determination of the proportion that is volatile on ignition.
8. Ether extractive.

The methods of analysis actually used on these 30 dye specimens are here given solely for the purpose of comparing them with the methods developed therefrom and presented beginning with page 210. Experience has shown that the methods here given are defective in many particulars, and therefore they are not to be used for exact work.

MOISTURE.

Dry a sample of each color weighing 3 grams at 105° to 108° C. for two hours. The loss in weight is assumed to be moisture.

This method is not wholly accurate in the case of Naphthol Yellow, nor is it accurate in the case of Amaranth; but the scarcity of material made it seem unwise, at this stage, to undertake any extended investigation as to the amount or nature of the heating required surely to expel all moisture. The results, therefore, while not as accurate as might be desired, are, for the purposes of this exploratory investigation, sufficiently accurate for the object for which they were undertaken.

CHLORIN AS CHLORIDS.

Gently heat samples weighing 0.1 gram with 2 grams of sodium carbonate, and after destroying the greater part of the organic matter add 0.1 gram of powdered potassium nitrate and gently heat the whole until the organic matter is entirely destroyed. After cooling treat the whole with small amounts of cold water and remove from the crucible; effect the solution of the whole by gently heating. After cooling bring the bulk to about 150 cc, cool to room temperature, slightly acidify with nitric acid, precipitate the chlorin with silver nitrate, and weigh as silver chlorid.

SULPHATED ASH.

Moisten half-gram samples with concentrated sulphuric acid, gently evaporate to dryness, and treat the residue with 5 cc of concentrated sulphuric acid and again evaporate to dryness; repeat the operation until a white ash results, when the whole is ignited to constant weight. After weighing take up the sulphated ash in boiling water (and if necessary, any undissolved material can be taken up with hydrochloric acid by treatment on a boiling-water bath.) Mix the two solutions and bring to a total volume of 200 cc. In one half thereof determine the contained sulphur by a precipitation with barium chlorid, and weigh as barium sulphate; in the other half determine iron and aluminum by precipitation with ammonia, ignite and weigh as ferric and aluminum oxid. No separation of any contained aluminum was undertaken in the filtrate from the iron; the lime was precipitated as oxalate. In the only case that copper was encountered it was precipitated as sulphid before taking out the iron or the calcium.

TOTAL SULPHUR.

Mix 0.2 gram of the sample with 4 grams of sodium carbonate and 0.5 gram of potassium nitrate, and ignite to complete the destruction of the organic matter; take up in hot water; acidify with hydrochloric acid and precipitate the sulphur as barium sulphate with barium chlorid.

GUTZEIT TEST.

Mix 2 grams of the substance with 2 grams of a mixture of 1 part of potassium nitrate and 5 parts of sodium carbonate and ash in a porcelain crucible over a low flame; if not white when cool, mix the ash with 1 gram of potassium nitrate and again ash over a low flame. Generally the second ashing is sufficient. Dissolve the residue in 50 cc of hot water, boil, filter, neutralize with dilute sulphuric acid, and evaporate to substantial dryness on a boiling-water bath. Then add 1 cc of concentrated sulphuric acid and dry over a Bunsen flame; take up the residue with 5 cc of distilled water containing 0.5 cc of concentrated sulphuric acid and 10 cc of a saturated solution of sulphurous acid; evaporate the whole to a bulk of 5 cc on a water bath; add 20 cc of 8 per cent hydrochloric acid and subject this material to the action of 2 grams of metallic zinc, free from arsenic, which has been so activized by means of platinic chlorid that at the end of two hours more than 1 gram of zinc has dissolved and the evolution of gas has been constant and continuous. Conduct the reaction in a flask of 60 cc capacity with a neck 1 cm in diameter and 6 cm long. After the introduction of the solution and the zinc into the flask, stopper the neck of the bottle with gauze, the lower half of which is dry and the upper half moistened with the test solution of lead acetate of the United States Pharmacopœia. After carefully wiping the lip of the flask, cover it with Schleicher & Schüll quantitative filter paper which has been saturated three times with alcoholic mercuric chlorid solution, with complete drying between each saturation on one and the same spot of the filter paper.

In the case of Naphthol Yellow S it is necessary to heat gently with 10 grams of sodium carbonate until the organic matter is substantially all destroyed, then add 1.5 grams of potassium nitrate and heat to complete destruction of the organic matter. Dissolve the fused mass in hot water and a few drops of fifth-normal sulphuric acid; then make distinctly acid with the same and add an excess of 1 cc of concentrated sulphuric acid; evaporate first on the water bath and afterwards on asbestos until all odor of nitrous fumes and of hydrochloric acid has disappeared. Take up the residue in 5 cc of water and 15 cc of a saturated solution of sulphurous acid in water. Heat the whole on the water bath until no odor of sulphur dioxid remains.

The preparation of the sample by ignition with carbonate and nitrate should not be done in platinum, but should be done in a porcelain crucible, since it has happened that as much as 0.05 mg of arsenic mixed with the dye, which had been fused in platinum with carbonate and nitrate, could not be detected on the mercury-chlorid paper, whereas 0.01 mg when similarly treated in porcelain could always be detected, and 0.005 mg would usually be found when done in porcelain.

HEAVY METALS.

Mix as much of the substance as approximately contains 1 gram of color with 10 times its weight of carbonate of soda and ignite with the addition of 0.5 gram of potassium nitrate. Dissolve the whole in water, any undissolved material being taken up with hydrochloric acid, bring the two solutions together and slightly acidify with hydrochloric acid; dilute to 100 cc; place 10 cc in a test tube of 40 cc capacity and warm to 50° C. in a water bath; add 10 cc of a freshly prepared saturated solution of hydrogen sulphid in water, stopper the test tube well and allow the whole to stand in water having a temperature of 35° C. for a half hour. Dilute a 3.3 cc portion to 10 cc, treat as before with 10 cc of hydrogen sulphid in water for one-half hour at 35° C., and add to each ammonium hydrate.

TOTAL INSOLUBLES.

Dissolve 1 gram of the substance in 1 liter of water, filter through counterpoised quantitative filters, and wash with hot water until all traces of color are removed from the filter paper, then dry at 100° C. to constant weight and weigh; report the weight as total insolubles. Ignite the total insolubles in a platinum crucible and report the residue remaining as nonvolatile insolubles.

ETHER EXTRACTIVE.

Two methods were employed, the one consisting in direct extraction of the substance in a Soxhlet apparatus by means of redistilled ether dried over sodium. Schleicher & Schüll extraction cartridges were used after they had been thoroughly extracted by ether and shown by examination that they yielded no extractive to ether whatever. This is a necessary precaution, because the amount of ether extractive matter in these cartridges varies. The amount of ether extractive was determined by driving off the ether over a 32-candlepower incandescent lamp, finally drying and cooling in a desiccator.

This method, however, is not satisfactory, since the results it gives are undoubtedly low and it seems that the higher the material was dried the more erratic were the results. Therefore the following method was used:

Disolve 1 gram of the sample in 100 cc of water; add 0.5 gram of sodium acetate; extract three times with 50 cc of ether in a separatory funnel; mix the ether so recovered with 10 cc of water; separate the ether and dry with fused calcium chlorid; let stand from 12 to 24 hours; pour off from the calcium chlorid; distil off the ether as in the preceding method; acidify the color solution containing sodium acetate with 1 cc concentrated hydrochloric acid; extract three times with 50 cc of ether, and proceed as before.

RESULTS OF CHEMICAL EXAMINATION, 1907.

DETAILED CHEMICAL DATA ON EACH PERMITTED COLOR.

The results of this examination are given in the following tabulations under the respective Green Table numbers; the serial numbers refer to the numbers assigned to the specimens as they were received. The abbreviations "p." and "n. p." opposite "Gutzeit test," and "Heavy metals test" stand for "pass," and "not pass," respectively, according as the specimen did or did not comply with those requirements; the entries opposite "Ratio," indicate the degree of concordance of the sulphated ash as weighed, and the contained SO_3 calculated back to sodium sulphate and is a measure of the accuracy or dependability of the sulphated ash item and its determination.

As a guide in ascertaining the conformity of these colors to their supposed standards when judged by the analytical data obtained, the following table may of service:

Percentages of sulphur, sodium, and sulphated ash properly belonging to each of the seven permitted colors.

Green Table No.	Name of colors.	Sulphur.	Sodium.	Sulphated ash.
4	Naphthol Yellow	8.95	12.87	39.73
56	Ponceau 3 R	12.97	9.33	28.80
85	Orange I	9.16	6.58	20.31
107	Amaranth	15.92	11.44	35.32
435	Light Green	11.12	8.31	25.64
517	Erythrosin	[1]	5.24	16.17
692	Indigotin	13.75	9.89	30.52

[1] Iodin in No. 517—57.7 per cent.

Detailed analytical data obtained on several samples of each of the permitted colors (per cent).

GREEN TABLE NO. 4. NAPHTHOL YELLOW S.

Determinations.	Serial Nos.				
	23.	73.	108.	142.	187.
Moisture	0.70	0.28	0.28	0.60	0.33
NaCl	6.73	3.47	1.02	.61	2.86
Na as NaCl	2.65	1.37	.40	.24	1.13
Sulphated ash:					
Al and Fe	.07	.07	.07	.07	.28
Ca	.04	None.	None.	None.	None.
Na_2SO_4	38.00	48.8	47.1	51.4	49.2
SO_3 in Na_2SO_4—Na_2SO_4	38.18	49.57	47.32	51.76	49.44
Ratio	100.3	101.6	100.5	100.7	100.5
Na as Na_2So_4	12.32	15.82	15.46	16.66	15.95
Total sulphur	8.72	9.47	12.63	12.42	12.32
Gutzeit test	n.p.	n.p.	n.p.	n.p.	n.p.
Heavy metals test	n.p.	n.p.	n.p.	n.p.	n.p.
Total insolubles	.05	None.	None.	None.	.05
Nonvolatile	.03	None.	None.	None.	None.
Volatile	.02	None.	None.	None.	.05
Acetate ether extract	.34	.15	.14	.16	.10
Acid ether extract	.98	.90	.70	.75	.32
Total ether extract	1.32	1.05	.84	.91	.42
Ether extract solid	.062	.042	.04	.03	.022

Determinations.	Serial Nos.				
	201.	209.	228.	272.	280.
Moisture	0.50	0.60	0.32	2.12	9.07
NaCl	23.67	4.08	2.86	3.26	9.39
Na as NaCl	9.31	1.61	1.13	1.28	3.70
Sulphated ash:					
Al and Fe	.21	None.	.07	.07	.07
Ca	.03	None.	None.	None.	.07
Na_2SO_4	49.20	53.10	55.60	45.60	40.20
SO_3 in Na_2SO_4—Na_2SO_4	49.75	53.76	56.45	45.80	40.14
Ratio	101.1	101.3	101.5	100.4	99.84
Na as Na_2SO_4	15.95	17.22	18.03	14.79	13.03
Total sulphur	6.35	12.56	13.42	10.50	7.55
Gutzeit test	n.p.	n.p.	n.p.	p.	p.
Heavy metals test	n.p.	n.p.	n.p.	n.p.	n.p.
Total insolubles	.30	.80	.12	.90	1.30
Nonvolatile	.20	None.	.10	None.	.04
Volatile	.10	80	.02	.90	1.16
Acetate ether extract	.14	.25	.08	.15	.10
Acid ether extract	.33	1.08	.90	.72	.77
Total ether extract	.47	1.33	.98	.87	.87
Ether extract solid	.10	.004	.13	.04	.02

Detailed analytical data obtained on several samples of each of the permitted colors (per cent)—Continued.

GREEN TABLE NO. 56. PONCEAU 3R.

Determinations.	Serial No. 9.	Determinations.	Serial No. 9.
Moisture	5.76	Ratio	101.20
NaCl	19.89	Na as Na_2SO_4	12.36
Na as NaCl	7.84	Gutzeit test	p.
Sulphated ash:		Heavy metals test	n. p.
Al and Fe	.28	Acetate ether extract	.28
Ca	None.	Acid ether extract	.83
Na_2SO_4	38.10	Total ether extract	1.11
SO_3 in Na_2SO_4—Na_2SO_4	38.56	Ether extract solid	.326

GREEN TABLE No. 85. ORANGE I.

Determinations.	Serial Nos.		Determinations.	Serial Nos.	
	16.	224.		16.	224.
Moisture	11.06	5.39	Total sulphur	10.12	8.34
NaCl	8.78	3.51	Gutzeit test	p.	n.p.
Na as NaCl	3.18	1.38	Heavy metals test	p.	p.
Sulphate ash:			Total insolubles	1.50	.55
Al and Fe	.14	.07	Nonvolatile	.20	.20
Ca	None.	.14	Volatile	1.30	.35
Na_2SO_4	30.80	18.00	Acetate ether extract	.02	.62
SO_3 in Na_2SO_4—Na_2SO_4	31.18	18.09	Acid ether extract	.20	.20
Ratio	101.3	100.50	Total ether extract	.22	.82
Na as Na_2SO_4	9.99	5.84	Ether extract solid	.214	.254

GREEN TABLE No. 435. LIGHT GREEN SF YELLOWISH.

Determinations.	Serial Nos.			
	57.	92.	168.	233.
Moisture	8.32	5.08	5.04	4.15
NaCl	.041	.102	1.735	1.530
Na as NaCl	.016	.040	.683	.608
Sulphate ash:				
Al and Fe	.70	.28	.14	.14
Ca	3.29	.00	.085	.085
Na_2SO_4	9.00	43.40	48.00	53.00
SO_3 in Na_2SO_4—Na_2SO_4	7.78	43.96	46.68	53.62
Ratio	86.46	101.30	95.92	101.3
Na as Na_2SO_4	2.92	14.07	15.56	17.19
Total sulphur	11.48	15.44	15.82	16.75
Gutzeit test	Tr. (p.)	Tr. (p.)	Heavy.	Heavy.
Heavy metals test	p.	p.	p.	p.
Total insolubles	.20	.45	.75	.95
Nonvolatile	None.	.06	.60	.60
Volatile	.20	.39	.15	.35
Acetate ether extract	.02	.02	.05	None.
Acid ether extract	.05	.05	.05	.05
Total ether extract	.07	.07	.10	.05
Ether extract solid	.026	None.	.02	.02

GREEN TABLE No. 107. AMARANTH.

Determinations.	Serial Nos.					
	82.	96.	130.	162.	177.	219.
Moisture	4.24	6.46	6.23	9.81	4.20	8.24
NaCl	38.91	12.79	35.79	25.39	28.45	24.21
Na as NaCl	15.32	5.04	14.10	10.00	11.21	9.54
Sulphate ash:						
Al and Fe	.14	.07	.14	.42	.42	.21
Ca	.215	.857	.93	.428	.428	.57
Na_2SO_4	65.20	53.20	59.80	48.80	60.00	49.80
SO_3 in Na_2SO_4—Na_2SO_4	66.50	55.60	60.30	49.52	61.26	50.72

Detailed analytical data obtained on several samples of each of the permitted colors (per cent)—Continued.

GREEN TABLE No. 107. AMARANTH—Continued.

Determinations.	Serial Nos.					
	82.	96.	130.	162.	177.	219.
Ratio	102.00	100.80	100.80	101.5	102.1	101.9
Na as Na_2SO_4	21.13	17.22	19.39	15.82	19.45	16.14
Total sulphur	8.62	13.02	8.57	9.42	11.09	8.54
Copper	None.	None.	None.	.09	None.	None.
Gutzeit test	n. p.	n. p.	n. p.	n. p.	n. p.	n. p.
Heavy metals test	n. p.	n. p.	n. p.	n. p.	n. p.	n. p.
Total insolubles	.30	1.50	2.05	1.05	.90	2.40
Nonvolatile	.12	1.10	1.10	.35	.35	1.15
Volatile	.18	.40	.95	.70	.55	1.25
Acetate ether extract	.08	.15	.18	.20	.02	.30
Acid ether extract	.07	.15	.15	.03	.03	.10
Total ether contract	.15	.30	.33	.23	.05	.40
Ether extract solid	.024	.062	.052	.040	.044	.054

GREEN TABLE NO. 517. ERYTHROSIN.

Determinations.	Serial Nos.			
	184.	200.	216.	254.
Moisture	....	....	9.6	....
NaCl (not determined)	....	....	....	....
Sulphated ash:				
Al and Fe	0.14	0.14	.14	0.28
Ca	None.	None.	None.	None
Na_2SO_4	39.00	35.69	31.80	26.4
SO_3 in Na_2SO_4=Na_2SO_4	39.44	35.44	32.46	26.8
Na as Na_2SO_4	12.65	11.52	10.40	8.56
Gutzeit test	p.	n. p.	p.	p.
Heavy metals test	p.	p.	p.	p.
Total insolubles	.60	.20	.65	.90
Nonvolatile	.04	None.	.20	.20
Volatile	.56	.20	.45	.70
Acetate ether extract	.80	.35	.10	.10
Total ether extract	....	....	....	....
Ether extract solid	.024	.07	.02	.02

GREEN TABLE NO. 692. INDIGO DISULPHO ACID.

Determinations.	Serial Nos.			Determinations.	Serial Nos.		
	90.	195.	249.		90.	195.	249.
Moisture	7.25	5.32	7.31	Total sulphur	10.30	10.30	10.09
NaCl	7.02	28.66	7.02	Gutzeit test	p.	p.	p.
Na as NaCl	2.77	11.29	2.77	Heavy metals test	p (?)	p (?)	p (?)
Sulphated ash:				Total insolubles	1.55	.80	1.15
Al and Fe	.42	.28	.56	Nonvolatile	.55	None.	.50
Ca	.57	.14	.71	Volatile	1.05	.80	.65
Na_2SO_4	26.20	55.40	26.60	Acetate ether extract	.02	.02	.02
SO_3 in Na_2SO_4=Na_2SO_4	26.74	56.64	27.36	Acid ether extract	.05	.10	.03
Ratio	102.10	102.20	102.8	Total ether extract	.07	.12	.05
Na as Na_2SO_4	8.50	17.97	8.63	Ether extract solid	.002	.014	.02

RECALCULATION OF ANALYTICAL DATA ON BASIS OF COLORING MATTER PRESENT.

The difficulties in the way of translating these analytical data into proximate constituents are so great, in so many of the cases, as to make any attempts to obtain practical results in that way absolutely useless; the amount of material on hand was unfortunately so small at the beginning (2 ounces or less, in most instances) that the utmost economy of material was necessary to get the data reported, on account of the large amount of material needed in the exploratory work done in trying out the methods for determining arsenic, heavy metals, and ether extractives.

In the arsenic test the coloring matter, as a whole, was considered, and no attempt was made to get at the actual amount of real coloring matter in the exact weights of the material as a whole, used for such examination, and consequently the results are not translatable into actual weights of real color used. It is obvious that if 2 grams, or other weight of the substance as a whole, failed to pass any test when the whole amount was considered as though it were all color, the material could not possibly have passed those tests if amounts thereof, corresponding to the prescribed weights of actual color used, were taken; also it is clear that if the sample, as a whole, passed a given test, at a given weight thereof, it would not necessarily have passed that test had an amount thereof, corresponding to that same weight of actual color, been used.

The amount of actual or real coloring matter in 27 of these 30 specimens is not greater than shown in the following:

Per cent of actual coloring matter in 27 samples.

Name of color.	Serial No.	Per cent.	Serial No.	Per cent.
Naphthol Yellow S	23	91.20	201	75.56
	73	95.20	209	93.19
	108	97.86	228	95.72
	142	97.88	272	92.81
	187	96.34	280	79.47
Ponceau 3R	9	73.24	....	....
Orange I	16	78.44	224	89.76
Amaranth	82	56.40	162	63.52
	96	78.95	177	66.40
	130	55.60	219	64.25
Light Green SF Yellowish	57	91.37	168	92.37
	92	94.30	233	93.32
Erythrosin	216	89.65	....	....
Indigo Disulpho Acid	90	84.11	249	84.47
	195	65.10	....	....

In order to make these analytical results comparable among themselves and with other analytical results later to be given, the items common salt, volatile and nonvolatile insolubles, total insolubles, acetate ether extract, acid ether extract, and total ether extract of the preceding tabulations have been recalculated in parts per 100 of actual or real coloring matter as just enumerated:

Analytical data recalculated to parts per hundred of coloring matter present.

GREEN TABLE NO. 4. NAPHTHOL YELLOW S.

Determinations.	Serial Nos.									
	23.	73.	108.	142.	187.	201.	209.	228.	272.	280.
Common salt	7.38	3.64	1.04	0.62	2.97	31.53	4.38	2.99	3.51	11.82
Insolubles:										
Volatile	.02	.00	.00	.00	.05	.13	.86	.02	.97	1.46
Nonvolatile	.03	.00	.00	.00	.00	.27	.00	.10	.00	.05
Total	.05	.00	.00	.00	.05	.40	.86	.12	.97	1.51
Ether extract:										
Acetate	.37	.16	.14	.16	.10	.19	.27	.08	.16	.13
Acid	1.07	.95	.72	.77	.33	.44	1.16	.94	.78	.97
Total	1.44	1.11	.86	.93	.43	.63	1.43	1.02	.94	1.10

GREEN TABLE NO. 56. PONCEAU 3R.

Determinations.	Serial No. 9.	Determinations.	Serial No. 9.
Common salt	27.16	Ether extract:	
Insolubles:		Acetate	0.38
Volatile	....	Acid	1.13
Nonvolatile	....	Total	1.51

GREEN TABLE NO. 85. ORANGE I.

Determinations.	Serial Nos.		Determinations.	Serial Nos.	
	16.	224.		16.	224.
Common salt	11.19	3.91	Ether extract:		
Insoluble:			Acetate	0.02	0.69
Volatile	1.66	.39	Acid	.25	.23
Nonvolatile	.25	.23	Total	.27	.92
Total	1.91	.62			

GREEN TABLE NO. 107. AMARANTH.

Determinations.	Serial Nos.					
	82.	96.	130.	162.	177.	219.
Common salt	68.89	16.20	64.37	39.97	42.85	37.39
Insolubles:						
Volatile	.32	.51	1.71	1.10	.83	1.93
Nonvolatile	.21	1.39	1.98	.55	.54	1.78
Total	.53	1.90	3.69	1.65	1.37	3.71
Ether extract:						
Acetate	.14	.19	.32	.31	.03	.46
Acid	.12	.19	.27	.05	.05	.15
Total	.26	.38	.59	.36	.08	.61

Analytical data recalculated to parts per hundred of coloring matter present—Continued.

GREEN TABLE NO. 435. LIGHT GREEN SF YELLOWISH.

Determinations.	Serial Nos.			
	57.	92.	168.	232.
Common salt	0.04	0.10	1.88	1.64
Insoluble:				
Volatile	.22	.41	.16	.38
Nonvolatile	.00	.06	.65	.64
Total	.22	.47	.81	1.02
Ether extract:				
Acetate	.02	.02	.05	.05
Acid	.05	.05	.05	.05
Total	.07	.07	.10	.10

GREEN TABLE NO. 517. ERYTHROSIN.

Determinations.	Serial No. 216.	Determinations.	Serial No. 216.
Common salt	....	Ether extract:	
Insoluble:		Acetate	0.11
Volatile	0.50	Acid	....
Nonvolatile	.22	Total	.11
Total	.72		

GREEN TABLE NO. 692. INDIGO SULPHO ACID.

Determinations.	Serial Nos.			Determinations.	Serial Nos.		
	90.	195.	249.		90.	195.	249.
Common salt	8.35	44.02	8.31	Ether extract:			
Insoluble:				Acetate	0.02	0.03	0.02
Volatile	1.25	1.23	.77	Acid	.06	.15	.04
Nonvolatile	.59	.00	.59	Total	.08	.18	.06
Total	1.84	1.23	1.36				

MARKET QUALITY OF THE SEVEN PERMITTED COLORS.

That the quality of the lots of the seven permitted colors of Food Inspection Decision No. 76 offered on the markets of the world after the issuance of that decision was no better than that of the lots just reported, if as good, appears from the paper of E. G. Kohnstamm entitled, "Certified Food Colors: The Difficulties in the Way of their Manufacture," presented to the Seventh International Congress of Applied Chemistry held in London, May and June, 1909, and published in abstract form. This abstract reads as follows:

> The coal-tar colors permitted under the food and drugs act of the United States are seven in number, which must be in a high state of purity. The author states that none of the colors on the markets of the world, at the time of testing, would meet these requirements.

Of these seven colors, 189 samples, from every possible source, and representing all the leading manufacturers, are here reported on, and the results of their examination reasonably establish the necessity of food color certification. Of these 189 samples, the worst were offered for food coloring purposes and seemed to be so offered because unfit for any other purpose.

Naphthol Yellow S.—Sixty-four samples examined, ranging in shade from clear bright yellow to a dirty brownish or green color. All contained Martius Yellow, some more than 1 per cent thereof; some contained as high as 2 per cent and even 3 per cent unconverted initial material or decomposition products. Forty-one contained excessive arsenic and 29 excessive heavy metals.

Orange I.—Twenty-eight samples examined; all contained decomposition products varying from a slight amount to over 50 per cent; free α-naphthol was found in most samples; in 12 it was as high as 2 per cent; insoluble matters were as high as 1 per cent; lead to the extent of 0.5 per cent was found in one sample; shading by added colors and excessive amounts of arsenic; lead and iron were frequent.

Amaranth.—Thirty-eight samples examined; none were pure, and all contained arsenic in excessive amounts, and in one case as high as 0.1 per cent; all contained added color, principally an acid-violet. Iron as high as 0.1 per cent; insoluble matter as high as 1½ per cent; was most heavily loaded with salt of all seven colors.

Ponceau 3R.—Thirty-six samples examined; the purest of all colors tested; not toned; heavily loaded with salt; much insoluble matter was present; decomposition products were absent; iron, 0.01 per cent; 12 contained excessive amounts of arsenic and 14 contained excessive amounts of heavy metals.

Erythrosin.—Twelve samples examined; 10 were not erythrosin at all; of the other two, one was low in iodin and one contained arsenic.

Light Green S F Yellowish.—Thirteen samples examined; only one free from arsenic; nine contained lead or copper; one contained manganese; none were loaded.

Indigo Disulphonic Acid.—Eight samples examined; none were pure; the iron content was as high as 1.5 per cent; all were loaded with salt or Glauber's salt. One contained excessive amount of arsenic and two excessive amounts of heavy metals.

The difficulties consist in keeping the undesirable materials out of the dyes or in separating them from the crude dyes, or both.

In this connection the following statement made by Dr. E. Ludwig, of Vienna, at the International Congress of Medicine held in Budapest, August, 1909, may be of interest:

The author, at an order of a court, at the beginning of the seventies in the last century, examined approximately 200 samples of food products confiscated as suspicious and taken from numerous sales places of a then suburb of Vienna; these samples included solid confectionery, fruit sirups, spirits, etc. He found that more than 90 per cent of these things were colored with magenta and contained arsenic. In some of the sales places the preparation used for coloring, the so-called "couleur," was found, which proved to be a solution of magenta and in which there were contained 8 per cent of arsenic in the form of arsenous acid and of arsenic acid. According to the statements of a qualified dyestuff maker, this "couleur" was a mother liquor from magenta manufacture, which was very difficultly saleable and which, however, a conscienceless agent had talked onto ignorant producers of and dealers in foods.

Schacherl (*p. 1046*) says: "It should be required of all permitted coloring matters that they shall not contain substances which are harmful to health, or even suspicious, either in chemical union or as contaminations." The following pages (Section XV) show how closely this requirement has been met as a result of quality control on the part of the Department of Agriculture.

XV. GUIDES IN DETERMINING DEGREE OF PURITY AND CLEANLINESS.

In view of the absence of any statements in the literature defining the purity of the colors physiologically examined with such accuracy that another could obtain with reasonable certainty the same degree of cleanliness, it became necessary to devise some guide, no matter how empirical, in the setting up of standards, tentative or otherwise.

As a first consideration it was held that, in view of the fact that all of the physiological work had been done with specimens of coal-tar colors of commercial purity, it would be reasonable to suppose that coal-tar colors produced in the purest form possible with present-day methods would certainly be as clean and as free from admixture as any of the commercial products subjected to physiological test. It was considered unreasonable to expect that increasing the degree of purity of these substances could in any way increase any harmful property possessed by them. Certainly in the case of Naphthol Yellows, where Martius Yellow is a usual contaminant, it can hardly be maintained that decreasing the amount of Martius Yellow would increase any harmful property which might reside in Naphthol Yellow S proper; in the case of Ponceau 3 R it could hardly be argued that any undisazotized base or decomposition products of diazo compounds tended to correct or counteract any harmful property that might reside in Ponceau 3 R proper; nor could it be maintained that Orange I free from uncombined alpha-naphthol was more harmful than Orange I, contaminated with alpha-naphthol, and so on through the list of the seven permitted colors. This point would not be raised had it not been pressed repeatedly by different persons as a serious objection to quality control and purity standards of the seven permitted colors of Food Inspection Decision No. 76.

With this rule in mind, and referring to the tabulated results of the analyses of the 30 specimens of the seven permitted colors just given, the items in the analytical statements will each be separately discussed.

Common salt and ether extractive.—Common salt is a legitimate component of commercial brands of coal-tar colors in so far as these coal-tar colors are obtained by the so-called "salting out" process. The coal-tar colors are recovered from solution by the addition of common salt, which has the peculiar property of separating the coal-tar color from the water solution as an undissolved solid. The coal-tar color so obtained will contain more or less salt, which, from a commercial manufacturing point of view, is therefore an unavoidable constituent. The amount of salt so accompanying the coal-tar color depends upon the amount added to the color solution, and this is greatest when complete exhaustion of the coal-tar color solution is attempted. It is a matter of common experience that, as a rule, the coal-tar color first separating in the salting-out process is cleaner and less contaminated than the portions last separating. In an endeavor to recover as far as possible all the dissolved coal-tar coloring matter the manufacturer adds a large excess of salt, and this carries with it a large amount of organic matter not coloring matter, as can be seen from the preceding analyses, where a high salt content is almost always accompanied by a high ether extract content. The ether extract content is a measure of the dirt or organic impurities of the coloring matter.

The objection to an excessive amount of salt in coal-tar coloring matters, when they are to be used for food-coloring purposes, does not reside in the salt per se, but is due to the fact that a high salt content seems generally to be accompanied by an unnecessarily large amount of organic material not coloring matter, and nothing good is known of such material, which is more than likely to be harmful.

The acetate ether extract is supposed to represent that part of the organic material not coloring matter which has no, or only slightly, basic properties; the acid ether extract is supposed to represent that part of the organic material not coloring matter which has acid properties. In the case of Naphthol Yellow S the acid ether extract will contain all of the Martius Yellow, and very likely will consist substantially of it. In the case of Erythrosin, of course, no acid ether extraction was attempted because the color acid is itself ether-soluble.

The following tabulation shows the percentages of salt based on the amount of coloring matter present contained in 26 of the specimens before reported on, arranged under each of the seven permitted colors:

Naphthol Yellow S: 0.62; 1.04; 2.97; 2.99; 3.51; 3.64; 4.38; 7.38; 11.82; 31.53. (Average, 6.99.)
Ponceau 3 R: 27.16.
Orange I: 3.91; 11.19. (Average, 7.55.)
Amaranth: 16.20; 37.39; 39.97; 42.85; 64.37; 68.89. (Average, 44.95.)
Light Green SF Yellowish: 0.04; 0.10; 1.64; 1.88. (Average, 0.92.)
Indigo Disulpho Acid· 8.31; 8.35; 44.02. (Average, 20.23.)

The following figures give the same data for the acetate, acid, and total, ether extract:

Ether extractives.

ACETATE ETHER EXTRACT.

Naphthol Yellow S: 0.08; 0.10; 0.13; 0.14; 0.16; 0.16; 0.16; 0.19; 0.27; 0.37. (Average, 0.18.)
Ponceau 3 R: 0.38.
Orange I: 0.02; 0.69. (Average, 0.36.)
Amaranth: 0.03; 0.14; 0.19; 0.31; 0.32; 0.46. (Average, 0.24.)

Light Green SF Yellowish: 0.02; 0.02; 0.05; 0.05. (Average, 0.035.)
Erythrosine: 0.11.
Indigo Disulpho Acid: 0.02; 0.02; 0.03. (Average, 0.03.)

ACID ETHER EXTRACT.

Naphthol Yellow S: 0.33; 0.44; 0.72; 0.77; 0.78; 0.94; 0.95; 0.97; 1.07; 1.16. (Average, 0.81.)
Ponceau 3 R: 1.13.
Orange I: 0.23; 0.25. (Average, 0.24.)
Amaranth: 0.05; 0.05; 0.12; 0.15; 0.19; 0.27. (Average, 0.14.)
Light Green SF Yellowish: 0.05; 0.05; 0.05; 0.05. (Average, 0.05.)
Indigo Disulpho Acid: 0.04; 0.06; 0.15. (Average, 0.08.)

TOTAL ETHER EXTRACT.

Naphthol Yellow S: 0.43; 0.63; 0.86; 0.93; 0.94; 1.02; 1.10; 1.11; 1.43; 144. (Average, 0.99.)
Ponceau 3R: 1.51.
Orange I: 0.27; 0.92. (Average, 0.59.)
Amaranth: 0.08; 0.26; 0.36; 0.38; 0.59; 0.61. (Average, 0.38.)
Light Green SF Yellowish: 0.07; 0.07; 0.10; 0.10. (Average, 0.09.)
Erythrosin: 0.11.
Indigo Disulpho Acid: 0.06; 0.08; 0.18. (Average, 0.11.)

Insoluble matter.—The amount of insoluble matter (total, volatile, and nonvolatile) is a measure of the cleanliness of the materials used, as well as of the cleanliness of treatment during the manufacture of the coloring matter. The following tabulation shows the variations in these three figures for each of the 26 specimens of the 7 permitted colors, examined, as in the preceding cases:

Insoluble matter.

VOLATILE INSOLUBLE.

Naphthol Yellow S: 0.00; 0.00; 0.00; 0.02; 0.02; 0.05; 0.13; 0.86; 0.97; 1.46. (Average, 0.35.)
Orange I: 0.39; 1.66. (Average, 1.03.)
Amaranth: 0.32; 0.51; 0.83; 1.10; 1.71; 1.93. (Average, 1.07.)
Light Green SF Yellowish: 0.16; 0.38; 0.22; 0.41. (Average, 0.29.)
Erythrosin: 0.50.
Indigo Disulpho Acid: 0.77; 1.23; 1.25. (Average, 1.08.)

NONVOLATILE INSOLUBLE.

Naphthol Yellow S: 0.00; 0.00; 0.00; 0.00; 0.00; 0.00; 0.03; 0.05; 0.10; 0.27. (Average, 0.05.)
Orange I: 0.23; 0.25. (Average, 0.24.)
Amaranth: 0.21; 0.54; 0.55; 1.39; 1.78; 1.98; (Average, 1.08.)
Light Green SF Yellowish: 0.00; 0.06; 0.64; 0.65. (Average, 0.34.)
Erythrosin: 0.22.
Indigo Disulpho Acid: 0.00; 0.59; 0.59. (Average, 0.36.)

TOTAL INSOLUBLE.

Naphthol Yellow S: 0.00; 0.00; 0.00; 0.05; 0.05; 0.12; 0.40; 0.86; 0.97; 1.51. (Average, 0.40.)
Orange I: 0.62; 1.91. (Average, 1.27.)

Amaranth: 0.53; 1.37; 1.65; 1.90; 3.69; 3.71. (Average, 2.14.)
Light Green SF Yellowish: 0.22; 0.47; 0.81; 1.02. (Average, 0.63.)
Erythrosin: 0.72.
Indigo Disulpho Acid: 1.23; 1.36; 1.84. (Average, 1.48.)

Work subsequent to these analyses showed that the insoluble matter, particularly the nonvolatile insoluble matter, was greater in certain batches than in others, and that these amounts were in excess of the maximum amounts reported therefor. Examination showed that these increased amounts of insoluble matter were probably due to variations in the water delivered to the factories by their respective municipal water supplies, since it was noted that in two or more cases the batches containing an exceptionally large amount of nonvolatile insoluble matter were manufactured at a time when the city water supply was abnormally hard; this item, therefore, has been made in some instances a little more elastic than the preceding analyses would seem to justify.

Arsenic.—Examination of the preceding tabulations shows that 14 out of 30 specimens tested passed the Gutzeit test for arsenic, and 16 failed to pass.

Heavy metals.—Inspection of the preceding tables shows that 13 out of 30 specimens passed this test, and that 17 failed to pass.

Combined arsenic and heavy metals test.—Inspection of the preceding tables shows that 11 out of 30 passed both tests jointly; that 3 passed the arsenic test and failed to pass the heavy metals test; and that 2 passed the heavy metals test and failed to pass the arsenic test.

Moisture.—The amount of moisture, as inspection of the preceding analyses shows, is variable, and is a factor not under easy control. As long as the coloring matters submitted for foundation certification were in powder form and the analyses disclosed the actual percentage of coloring matter it was considered that any control of this item would involve an amount of labor and provide opportunities for friction wholly out of proportion to any benefit that at present could be realized therefrom. Therefore such control was not instituted, although it by no means follows that the time may not come when control of this item will be necessary.

Sulphur and sulphated ash.—These two determinations in the case of all the permitted colors, except Erythrosin, give a rough measure of the extent of the sulphonation and of the saturation of the sulphonic acids with sodium. It is not the function of these two items to exclude the isomeric modifications, nor was any test applied to such of these 30 specimens as were examined in this respect to determine the presence or absence of such products. The attempts made in this direction are of a later date than the analyses already reported, and are indicated on page 210 of this report.

With these data available, and with the general rule stated at the opening of this section in mind, it was regarded as the proper course, when determining upon standards, to require that each color should pass the combined heavy metals and arsenic test of the United States Pharmacopœia, and in other respects should be as clean and as high-grade as the best of each class given in the preceding analyses. Just how closely it was possible to adhere to this rule will be shown later.

With respect to the arsenic requirement, there has been a great deal of discussion brought on by those interested in the manufacture of these colors. It was protested that colors could not be made uniformly and continuously under manufacturing conditions, which would contain an amount of arsenic so small that a quantity of the product containing 2 grams of the coloring matter in question would not respond to the Gutzeit test in the United States Pharmacopœia. The results above given with respect to conformity or nonconformity to the Gutzeit test, were, with a great deal of justification, not regarded as conclusive, because, as has been previously stated, there was no certainty that the amount of coloring matter actually taken did represent 2 grams. In view of the fact that the United States Pharmacopœia prescribes for the only coal-tar color in it, namely, Methylene Blue, that it shall be so free from arsenic that 2 grams fail to respond to the Gutzeit test, it was considered perfectly justifiable to adhere to that requirement until it could be conclusively shown that it was unreasonable and incapable of attainment. Results described in the pages that follow show that it has been possible to make all the 7 permitted colors of Food Inspection Decision No. 76 so free from arsenic that they comply with the Pharmacopœial test.

With respect to the heavy metals, no deviation was necessary for the nonferrous metals from the test of the Pharmacopœia; for iron, however, it was found necessary to increase the limit to substantially 0.005 part per 100 of actual coloring matter. The reason for this is that at one time or another all of the seven permitted coloring matters in the course of their manufacture come in contact with, or are contained in, vessels of iron, and it seems to be almost impossible to keep iron out to an extent which would bring the color within the pharmacopœial test. It has been shown that there were 13 colors on the market in 1907 which contained so little iron that they failed to respond to the heavy metals test of the Pharmacopœia in that respect; but here again the same criticism holds good as in the arsenic test, that there is no certainty that the amounts taken for the heavy metals tests were equivalent in each case to 1 gram of actual coloring matter, and subsequent experience seems to confirm the correctness of that criticism; therefore the rule for cleanliness of product, as just given, has been modified in that respect and to that extent.

That it was only fair and reasonable to expect considerable improvement in the cleanliness and purity of food colors was made evident by an examination of two substances sold in the United States in large quantities for the purpose of making a very cheap red coal-tar coloring matter, which is used in many of the cheapest coloring operations, for paints, inks, etc. These substances are paranitranilin and betanaphthol. They were found in the United States market in such a condition of cleanliness and purity that had they been suitable for use in foods no objection could be raised against them on these scores. They complied with the requirements of the United States Pharmacopœia with respect to freedom from arsenic and all heavy metals, inclusive of iron. The significance of this lies in the fact that all the raw materials entering into the manufacture of paranitranilin and betanaphthol also enter into the manufacture of the seven permitted colors of Food Inspection Decision No. 76, and that the only source of arsenic in food colors, and probably the only way in which iron could be introduced into them, is by way of the materials entering into the manufacture of paranitranilin and betanaphthol, and since it has been shown to be commercially possible to keep those bodies out of paranitranilin and betanaphthol, and since there is no occasion whatever for arsenic or iron or other heavy metal being present in any of the materials used in the manufacture of the seven permitted colors of Food Inspection Decision No. 76, over and above the materials used in the manufacture of paranitranilin and betanaphthol, there was every reason for believing that the seven permitted colors of Food Inspection Decision No. 76 could ultimately be manufactured and marketed in the same degree of cleanliness and purity that paranitranilin and betanaphthol are marketed; in other words, that food colors could be made as clean and as pure as paint colors, a condition not existing in the food-color market of the United States in the summer of 1907.

The results of the control exercised by the Department of Agriculture over the quality of food colors, as compiled in the following section, fully justify such expectations, as well as the aim to make coal-tar colors when used for food purposes of the same high degree of cleanliness and purity as when they are to be used as drugs or as they actually are when used for the manufacture of paints and printer's inks; that is to say, that the coal-tar color used in a colored food should be as clean as the coal-tar color used in making the ink on the label of such colored food, the very reverse of the situation existing prior to the quality control now established.

XVI. ANALYSES OF CERTIFIED LOTS OF PERMITTED COLORS, 1909-10.

TABULATION OF RESULTS.

The analytical results obtained on 74 batches of certified colors, totaling upward of 32,000 pounds, are given in the following tables. The analytical results are the work of the New York Food and Drug Inspection Laboratory in the course of checking up the analytical results certified to in foundation and in supplementary certificates. The period covered by these examinations is approximately from July, 1909, to January 1, 1910.

Naphthol Yellow S.

[Figures calculated to 100 parts pure color and arranged in the order of their size. Figures on the same horizontal lines do not refer to the same samples; the dash line shows the location of the average of each column.]

Number of batches.	Insolubles.		Ether extractives.				Common salt.	Sulphur.	Sodium.	Calcium.
	Total.	Inorganic.	Neutral.	Alkali.	Acid.	Total.				
1	0.170	0.100	0.036	0.016	0.067	0.088	4.06	9.23	13.27	0.13
2	.130	.078	.026	.007	.065	.073	2.26	8.99	13.21	.13
3	.097	.050	.024	.006	.042	.072	1.68	8.98	12.92	.05
4	.090	.046	.023	.006	.038	.064	1.41	8.96	12.90	.04
5	.082	.040	.019	.005	.036	.061	1.30	8.89	12.89	....
6	.079	.036	.018	.005	.034	.057	.63	8.86	12.89	....
7	.070	.032	.016	.004	.031	.056	.63	8.85	12.87	....
8	.060	.030	.012	.004	.026	.054	.48	8.83	12.87	....
9	.055	.010	.011	.000	.025	.048	.47	8.71	12.81	....
10	.050	.010	.010	.000	.025	.045	.25	8.70	12.76	....
11	.050	....	.007	.000	.020	.045	.24	8.62	....	....
12	.030	....	.006	.000	.018	.045	.19	....	....	....
13	.030	....	....	....	....	.044	.19	....	....	....
Average	.076	.043	.017	.004	.035	.051	1.06	8.87	12.93	.09
Above	6	4	6	6	5	8	5	5	2	2
Below	7	6	6	6	7	5	8	6	8	2

Ponceau 3R.

[Figures calculated to 100 parts pure color and arranged in the order of their size. Figures in the same horizontal line do not refer to the same sample; the dash line shows the location of the average of each column.]

Number of batches.	Insoluble.		Ether extractives.				Common salt.	Sodium sulphate.	Sulphur.	Sodium.	Calcium.	Boiling point of cumidin.
	Total.	Inorganic.	Neutral.	Alkali.	Acid.	Total.						
												°C.
1	0.55	0.29	0.166	0.034	0.019	0.216	5.72	0.06	13.03	9.51	0.18	222-235
2	.42	.20	.163	.033	.018	.213	5.67	.06	12.99	9.49	.15	222-235
3	.41	.17	.161	.032	.018	.201	5.57	.00	12.95	9.31	.14	222-235
4	.27	.12	.072	.009	.018	.098	5.00	.00	12.88	9.31	.14	220-230
5	.17	.11	.053	.006	.017	.073	4.12	.00	12.80	9.23	.12	220-230
6	.17	.11	.052	.002	.017	.072	3.73	.00	12.80	9.16	.12	220-230
7	.16	.09	.038	.002	.016	.058	3.71	.00	12.77	9.12	....	220-229
8	.14	.07	.038	.000	.014	.057	3.03	.00	12.64	9.07	....	225-228
9	.10	....	....	....	....	....	2.53	.00	....	8.74	....	216-226
Average	.266	.15	.093	.015	.017	.124	4.34	.01	12.83	9.21	.14	221-231
Above	4	3	3	3	4	3	4	....	4	5	2	3
Below	5	5	5	5	4	5	5	....	4	4	4	6

Orange I.

[Figures calculated to 100 parts pure color, and arranged in the order of their size. Figures in the same horizontal line do not refer to the same sample; the dash line shows the location of the average of each column.]

Number of batches.	Insoluble.		Ether extractives.				Common salt.	Sodium sulphate.	Sulphur.	Sodium.	Calcium.
	Total.	Inorganic.	Neutral.	Alkali.	Acid.	Total.					
1..........	0.39	0.18	0.224	0.093	0.08	0.360	3.13	0.22	9.26	6.82	0.29
2..........	.38	.08	.220	.060	.077	.309	3.13	.10	9.23	6.76	.24
3..........	.28	.08	.210	.043	.067	.306	3.04	....	9.12	6.63	.24
4..........	.23	.05	.207	.035	.055	.300	2.36	....	9.10	6.62	.22
5..........	.23	.04	.181	.035	.048	.269	1.98	....	9.06	6.54	.05
6..........	.16	.04	.177	.020	.039	.267	1.91	....	9.06	6.48	.04
7..........	.15	.03	.174	.018	.036	.246	1.90	....	9.05	6.32	....
8..........	.11	.03	.124	.015	.029	.224	1.41	....	9.02	6.21	....
9..........	.10	.02	.107	.011	.016	.175	1.05	....	8.96	....	....
10..........	.05	....	.097	.011	....	.124	1.01	....	8.15	....	....
Average....	.21	.06	.172	.034	.050	.258	2.09	.03	9.00	6.55	.18
Above......	5	3	7	5	4	6	4	1	8	4	4
Below......	5	6	3	5	5	4	6	1	2	4	2

Amaranth.

[Figures calculated to 100 parts pure color and arranged in the order of their size. Figures on the same horizontal lines do not refer to the same samples; the dash line shows the location of the average of each column.]

Number of batches.	Insoluble.		Ether extractives.				Common salt.	Sulphur.	Sodium.	Calcium.
	Total.	Inorganic.	Neutral.	Alkali.	Acid.	Total.				
1....................	0.43	0.33	0.090	0.030	0.059	0.158	4.18	16.04	14.22	0.20
2....................	.39	.30	.088	.017	.017	.101	4.16	15.80	14.19	.19
3....................	.29	.19	.069	.009	.015	.100	3.83	15.63	12.10	.17
4....................	.23	.15	.047	.007	.014	.060	3.73	15.61	11.47	.14
5....................	.21	.10	.042	.007	.012	.056	3.67	15.59	11.43	.14
6....................	.13	.10	.035	.005	.012	.053	3.08	15.58	11.43	.13
7....................	.12	.06	.034	.005	.012	.052	3.02	15.57	11.42	.13
8....................	.12	.05	.034	.005	.010	.051	2.94	15.57	11.38	.12
9....................	.12	.05	.034	.005	.009	.048	2.70	15.56	11.33	.12
10....................	.11	.04	.031	.004	.008	.042	2.62	15.54	11.32	.12
11....................	.11	.04	.031	.004	.008	.042	2.21	15.52	11.31	.11
12....................	.11	.040	.031	.004	.007	.040	2.12	15.52	11.29	.09
13....................	.11	.036	.027	.004	.006	.039	2.12	15.52	11.27	.09
14....................	.092	.03	.026	.004	.006	.038	2.04	15.47	11.27	.09
15....................	.09	.03	.026	.003	.005	.038	1.94	15.43	11.23	.09
16....................	.09	.025	.025	.002	.005	.038	1.94	15.41	11.21	.08
17....................	.089	.02	.021	.001	.004	.035	1.91	15.41	11.18	.07
18....................	.08	.02	.019	.000	.003	.035	1.91	15.41	11.15	.00
19....................	.070	.02	.018	.000	.003	.029	1.23	15.40	11.14	....
20....................	.07	.02	.012	.000	....	....	1.22	15.12	11.10	....
21....................	.04	....	....	....	....	....	....	15.12	11.01	....
Average..............	.148	.083	.037	.006	.011	.056	2.63	15.51	11.59	.012
Above..............	5	6	5	5	7	4	9	13	3	7
Below..............	16	14	15	15	12	15	11	8	18	11

Light Green SF Yellowish.

[Figures calculated to 100 parts pure color and arranged in the order of their size. Figures in the same horizontal line do not refer to the same sample; the dash line shows the location of the average of each column.]

Number of batches.	Insoluble.		Ether extractives.				Common salt.	Sodium sulphate.	Sulphur.	Sodium.	Calcium.
	Total.	Inorganic.	Neutral.	Alkali.	Acid.	Total.					
1..........	0.07	0.034	0.058	0.011	0.011	0.076	4.15	0.00	12.69	8.03	4.52
2..........	.045	.02	.041	.007	.009	.054	.64	.00	12.27	7.99	.10
3..........	.03	.01	.034	.006	.008	.053	.515	.00	12.07	7.99	.09
4..........	.02	.01	.033	.000	.007	.042	.43	.00	11.88	7.94	.06
5..........	.02	.01	....	....	....	....	.37	.00	11.88	7.73	.05
6..........	.01	.01	....	....	....	....	....	.00	....	1.56	....
Average....	.033	.017	.041	.006	.008	.056	1.221	.00	12.16	6.87	.96
Above......	2	2	1	2	2	1	1	0	2	5	1
Below......	4	3	3	2	2	3	4	0	3	1	4

Erythrosin.

[Figures calculated to 100 parts pure color and arranged in the order of their size. Figures in the same horizontal line do not refer to the same sample; the dash line shows the location o the average of each column.]

Number of batches.	Insoluble.		Ether extractives.				Common salt.	Sodium sulphate.	Sodium.	Iodin.
	Total.	Inorganic.	Neutral.	Alkali.	Acid.	Total.				
1....................	0.13	0.09	0.039	0.017	....	0.051	1.13	0.00	5.42	57.45
2....................	.09	.04	.039	.009	....	.048	1.00	.00	5.35	56.46
3....................	.059	.024	.037	.008	....	.047	.48	.00	5.33	56.40
4....................	.05	.01	.034	.002	....	.037	.23	.00	5.29	56.07
5....................	.04	.01	.033	.002	....	.035	.09	.00	5.15	55.98
6....................	.04	.01	.033	.000	....	.035	.09	.00	5.11	55.97
7....................	.04	.01	.027	....	....	.027	.09	.00	3.85	55.94
8....................	.03	....	....	....	....	....	.09	.00	....	55.90
9....................	....	....	....	....	....	....	.036	.00	....	55.88
Average............	.059	.028	.035	.006	....	.040	.360	.00	5.07	56.23
Above..............	2	2	3	3	....	3	3	....	6	3
Below..............	6	5	4	3	....	4	6	....	1	6

Indigo disulpho acid.

[Figures calculated to 100 parts pure color and arranged in the order of their size. Figures on the same horizontal lines do not refer to the same samples; the dash line shows the location of the average of each column.]

Number of batches.	Insoluble.		Ether extractives.				Common salt.	Sodium-sulphate.	Sulphur.	Sodium.	Calcium.
	Total.	Inorganic.	Neutral.	Alkali.	Acid.	Total.					
1..........	0.50	0.28	0.140	0.087	0.099	0.320	6.77	16.12	13.45	10.13	0.45
2..........	.46	.26	.134	.061	.038	.217	3.60	10.22	13.43	9.90	.13
3..........	.45	.25	.102	.029	.026	.143	3.21	4.79	13.42	9.64	.13
4..........	.43	.24	.088	.015	.024	.141	2.89	.00	13.39	9.60	.05
5..........	.42	.22	.075	.013	.016	.126	1.85	.00	13.22	9.13	....
6..........	.32	.16	....	....	....	....	.40	.00	13.15	8.54	....
Average....	.43	.235	.108	.041	.041	.189	3.12	5.19	13.34	9.49	.19
Above......	3	4	2	2	1	2	3	2	4	4	1
Below......	3	2	3	3	4	3	3	4	2	2	3

COMPARISON WITH ANALYSES MADE IN 1907.

A comparison, as far as possible, of these figures with the corresponding data previously given for samples on the market in the summer of 1907 is made in the following table. The figures in this new table show the value of the fraction obtained by dividing the old average figure by its corresponding new average figure—that is, they show how many times greater the old average figures are than the corresponding new average figures. Italics indicate those items in which there has been a retrogression in the new figures as compared with the old; all the other figures represent an advance. It will be noted that comparisons are not made for Ponceau 3R and Erythrosin. This comparison was omitted because there was but one sample of Ponceau 3R examined and a partial analysis of one sample of Erythrosin in the old work.

Ratio of average figures of 1907 to those for the certified colors, 1909.

Color.	Salt.	Insoluble.			Ether extracts.		
		Total.	Volatile.	Nonvolatile.	Nonacid.	Acid.	Total.
Naphthol Yellow S	4.88	4.44	9.02	*0.67*	7.50	21.89	15.97
Orange	3.54	9.07	20.60	3.00	1.57	4.53	2.26
Amaranth	15.07	11.26	21.40	7.71	4.07	10.77	5.00
Green	*.66*	31.00	29.00	17.00	*.65*	6.25	1.67
Blue	8.05	3.29	5.4	1.56	*.15*	3.48	*.68*

These retrogressions are:

1. Salt in the Green.
2. Nonvolatile insolubles in the Yellow.
3. Nonacid ether extract in the Green.
4. Nonacid ether extract in the Blue.
5. Total ether extract in the Blue.

With respect to the first of these retrogressions there is this to be said: One lot of Green had apparently been purified by precipitation with salt, since it was in every other respect of a high quality—that is, it was free from arsenic and heavy metals within the pharmacopœial test, and its ether extractives were very satisfactory. The other lots of Green examined had apparently not been made in this manner.

With respect to the second retrogression, the probable explanation is that some of the lots were made during a period when the municipal water supply was excessively hard, as before explained. The remaining three retrogressions are probably due to the fact that the old methods of analysis used were not so accurate as the methods later employed and hereinafter described (pp. 223, 225).

It will be noted that in three of the six batches of Indigo disulpho acid reported there is no sodium sulphate; whereas in the other three batches the amount of this substance is as high as 16.12. The reason for this is that in the early stages of the work the results obtained by the manufacturers pointed in the direction of the impossibility of getting rid of all the sodium sulphate; but later and more extended work showed this conclusion to be an error. There is therefore no good reason for permitting sodium sulphate as a contaminant in this or any other of the seven products.

CONFORMITY OF ANALYTICAL DATA WITH THEORETICAL COMPOSITION.

The seven tables following, likewise based upon the 74 Government analyses, show the conformity or nonconformity, as the case may be, of these samples with their theoretical composition. The second and third columns show the percentage based on theory of the contained sulphur and sodium in the case of all colors except Erythrosin, where the figures for iodin are substituted for those of sulphur. The fourth column shows the actual ratio of sodium to sulphur or iodin, as the case may be, and the fifth column gives the percentage of the figure of column 4 based on the theoretical value which is given in parentheses at the head of columns 4 and 5. The summary gives the maximum, minimum, and average of each column. Taken as a whole the figures are fairly satisfactory and show a reasonable conformity of the actual product to theoretical requirements, although there seems to be considerable room for improvement, which no doubt can be achieved in time.

Percentages based on theoretical composition.

NAPHTHOL YELLOW S.

Number of batches.	Sulphur.	Sodium.	$\frac{Na}{S}=(1.4379)$.		Number of batches.	Sulphur.	Sodium.	$\frac{Na}{S}=(1.4379)$.	
			Value.	Per cent.				Value.	Per cent.
	Per cent.	*Per cent.*				*Per cent.*	*Per cent.*		
1	103.120	103.10	1.4377	99.983	10	98.880	101.06	1.4576	101.36
2	96.312	99.068	1.4791	102.860	11	98.658	101.22	1.4631	101.74
3	97.205	100.000	1.4792	102.870	12	100.330	100.15	1.4353	99.826
4	97.542	99.534	1.4707	102.270	13	100.100	100.00	1.4363	99.890
5	....	....	....	....					
6	....	....	....	....	Average	99.101	102.212	1.4578	101.378
7	98.992	....	....	....	Max	103.120	103.500	1.4792	102.87
8	99.331	100.15	1.4498	100.80	Min	96.312	99.068	1.4353	99.826
9	100.440	103.50	1.4692	102.18					

PONCEAU 3R.

Number of batches.	Sulphur.	Sodium.	$\frac{Na}{S}=(0.71905)$.		Number of batches.	Sulphur.	Sodium.	$\frac{Na}{S}=(0.71905)$.	
			Value.	Per cent.				Value.	Per cent.
	Per cent.	*Per cent.*				*Per cent.*	*Per cent.*		
1	97.454	93.617	0.69138	96.173	8	98.693	101.920	0.74296	103.33
2	....	98.927	....	....	9	99.308	101.700	.73679	102.48
3	100.460	97.749	.69992	97.351					
4	100.140	98.174	.70518	98.080	Average	99.256	98.738	.71648	99.144
5	98.458	97.095	.70936	98.662	Max	100.46	101.920	.74296	103.33
6	99.841	99.783	.71892	99.918	Min	97.454	93.617	.69138	96.173
7	98.693	99.783	.72730	101.16					

Percentages based on theoretical composition—Continued.

ORANGE I.

Number of batches.	Sulphur.	Sodium.	$\frac{Na}{S}=(0.71905)$.		Number of batches.	Sulphur.	Sodium.	$\frac{Na}{S}=(0.71905)$.	
			Value.	Per cent.				Value.	Per cent.
1	88.972	100.44	0.81226	112.97	8	98.471	85.901	0.70066	97.454
2	100.76	103.72	.73889	102.77	9	99.563	99.241	.71710	99.740
3	101.09	102.53	.73002	101.53	10	99.342	98.330	.71208	99.042
4	99.135	....	....	....					
5	99.135	....	....	....	Average	98.308	99.376	.72957	101.475
6	97.816	94.234	.69309	96.391	Max	101.09	103.72	.81226	112.97
7	98.799	100.610	.73243	101.90	Min	88.972	95.901	.69309	97.454

AMARANTH.

1	96.795	105.76	0.78519	109.21	14	97.800	98.863	0.72640	101.03
2	98.176	99.037	.72488	100.82	15	97.499	99.911	.73646	102.43
3	94.975	124.29	.94048	130.80	16	97.614	98.950	.72843	101.32
4	94.975	124.04	.93849	130.53	17	97.499	99.476	.73325	101.98
5	100.750	96.240	.68637	95.470	18	97.863	98.689	.72465	100.78
6	99.246	99.911	.72341	100.62	19	98.052	98.393	.72106	100.05
7	97.174	100.260	.72454	100.78	20	97.800	98.514	.72383	100.68
8	96.929	99.827	.74011	102.94	21	97.735	97.725	.71852	99.914
9	97.926	97.465	.71520	99.476					
10	96.975	97.990	.72744	101.18	Average	97.467	101.347	.74707	103.90
11	96.733	97.389	.72338	100.62	Max	100.750	124.29	.94048	130.80
12	97.487	98.514	.72615	100.99	Min	94.975	96.240	.68637	95.470
13	96.798	97.028	.72030	100.18					

LIGHT GREEN SF YELLOWISH.

1	102.85	....	....	....	6	104.50	95.547	0.65783	91.495
2	....	96.148	....	....					
3	109.87	93.018	0.60911	84.722	Average	105.26	95.498	.64827	90.167
4	106.23	96.629	.65358	90.911	Max	109.87	96.629	.67256	93.540
5	102.35	96.148	.67256	93.540	Min	102.85	93.018	.60911	84.722

ERYTHROSIN.

Number of batches.	Iodin.	Sodium.	$\frac{Na}{I_2}=(0.090855)$.		Number of batches.	Iodin.	Sodium.	$\frac{Na}{I_2}=(0.090855)$.	
			Value.	Per cent.				Value.	Per cent.
	Per cent.	*Per cent.*				*Per cent.*	*Per cent.*		
1	96.915	73.473	0.068873	75.805	8	96.879	102.09	.095741	105.37
2	97.051	100.94	.094497	104.00	9	97.886	103.43	.095995	105.65
3	99.593	98.277	.089639	98.663					
4	97.780	97.521	.090604	99.724	Average	97.952	96.777	.090204	99.146
5	97.209	....	....	....	Max	99.593	103.43	.095995	105.65
6	96.983	....	....	....	Min	96.879	73.473	.068873	75.805
7	97.035	101.71	.095229	104.81					

Percentages based on theoretical composition—Continued.

INDIGO DISULPHO ACID.

Number of batches.	Sulphur.	Sodium.	$\frac{Na}{S}=(0.71905)$.	
			Value.	Per cent.
	Per cent.	*Per cent.*		
1........	97.670	103.330	0.71482	99.423
2........	95.636	98.279	.69430	95.669
3........	97.600	103.760	.71832	99.910
4........	96.148	91.926	.64598	89.848
5........	97.382	106.560	.73934	102.830
6........	97.818	109.040	.75315	104.750
Average.	97.042	102.149	.71099	98.738
Max.....	97.818	109.040	.75315	104.750
Min.....	95.636	91.926	.64598	89.848

ARSENIC DETERMINATIONS ON 86 BATCHES.

During the course of this work it became necessary to determine the exact amount of arsenic contained in 86 of the various batches of certified colors; for this purpose the arsenic method of Seeker and Smith (see p. 212) was devised. These results are expressed in the number of parts of color containing one part of metallic arsenic (As). The numbers in parentheses indicate the number of specimens of the quality indicated.

Naphthol Yellow S (17 specimens).—770,000, 833,000, 1,250,000 (2), 1,428,000 (2), 1,666,000 (3), 2,000,000 (6), 3,333,000 (2).

Ponceau (15 specimens).—588,000, 625,000, 667,000, 714,000 (2), 769,000 (2), 1,000,000 (2), 1,111,000, 1,250,000, 1,666,000 (2), 2,000,000, 2,500,000.

Orange (7 specimens).—200,000, 250,000, 588,000, 1,000,000, 1,111,000, 1,429,000, 5,000,000.

Amaranth (27 specimens).—909,000, 1,000,000, 1,111,000, 1,250,000 (2), 1,438,000 (3), 2,000,000 (4), 2,500,000 (4), 3,333,000 (9), 5,000,000 (2).

Green (8 specimens).—166,000, 200,000, 370,000 (2), 500,000, 666,000, 770,000, 833,000.

Erythrosin (7 specimens).—2,000,000 (2), 5,000,000 (2), 10,000,000 (2), 20,000,000 (1).

Blue (5 specimens).—285,000, 666,000, 1,428,000, 3,333,000 (2).

The United States Pharmacopœia test for arsenic is sensitive to 0.005 mg of arsenious oxid (As_2O_3) which on a sample containing 2 grams of actual or real color would amount to one part of arsenious oxid in 400,000 of color; calculated to metallic arsenic, the basis employed in the foregoing, this would mean 528,000 parts of color for each one part of metallic arsenic.

There are, therefore, in the foregoing 86 lots of certified colors 8 which did not comply with that requirement, namely:

Orange (2 specimens).—200,000 and 250,000.

Green (5 specimens).—166,000; 200,000; 370,000 (2); 500,000.

Blue (1 specimen).—285,000.

The reason for this discrepancy is that in the preparation of the samples for analysis by the United States Pharmacopœia test a loss of arsenic ensued, which is avoided in the Seeker-Smith method now employed in making these determinations. Had the existence of this discrepancy been proven at the time the first analysis was made certification would have been denied to the eight lots above mentioned.

These results further show the position taken early in the work by several of the manufacturers—that a requirement of not more than 1 part of metallic arsenic in not less than 264,000 parts of coal-tar color could not be complied with on a commercial scale—to be untenable; on this basis only 4 out of the 86 lots examined would have been excluded. Further, the position of some manufacturers and dealers that the arsenic requirement ought, for practical manufacturing reasons, not to be more rigorous than one part of metallic arsenic in 26,400 parts of color, or 1 part of arsenious oxid (As_2O_3) per 20,000 parts of color, is not borne out by the data.

SUGGESTED REQUIREMENTS FOR CERTIFIED COLORS.

Although the material embodied in this report gives a very good idea of the composition and quality of substantially 30 different lots of permitted colors prior to the issuance of Food Inspection Decisions Nos. 76 and 77, and of 74 lots of certified colors, yet these data are hardly sufficient to furnish a basis for standards with which each color specimen must comply in detail. The fitness or unsuitability of any lot has been determined by the examination of the analytical data obtained thereon in the Food and Drug Inspection Laboratory at New York; such examination has been applied to the particular color under investigation with respect to its general relationship to the results theretofore achieved. If in some minor quality, as, for example, freedom from salt, the sample was not up to what had been previously accomplished, but in a major quality, as for example, ether extractive, it was equal to or better than what had been previously accomplished, and the pharmacopœial tests for arsenic and heavy metals were satisfied with the exception of iron, and the amount of iron was within the limit previously stated, 0.005 per cent, and the other factors showed a fairly close conformity, such a defect as its salt content would not act as a bar to the passing of the lot; however, no matter how good a color might be in respect to such determinations as ether extractive, if it failed to comply with the United States Pharmacopœia requirement for arsenic or for heavy metals it was not accepted.

These arsenic results have been tabulated to show the distribution of arsenic content (As_2O_3); the numbers at the top are the Green Table numbers; the numbers in the body of the table indicate the number of specimens of the arsenic content stated in the first column; the last column shows the totals of all colors of the arsenic content (As_2O_3) corresponding thereto.

Arsenic content of 86 lots of certified colors.

Arsenic content.	Green Table numbers.							Total.
	4	56	85	107	435	517	692	
1 part in—								
[1] 126,200	......	......	......	......	[1] 1	......	......	1
[1] 151,000	......	......	......	......	[1] 1	......	......	1
[1] 151,500	......	......	[1] 1	......	......	......	......	1
[1] 189,300	......	......	[1] 1	......	......	......	......	1
[1] 215,900	......	......	......	......	......	......	[1] 1	1
[1] 280,300	......	......	......	......	[1] 2	......	......	2
[1] 378,700	......	......	......	......	[1] 1	......	......	1
445,400	......	1	1	......	......	......	......	2
473,500	......	1	......	......	......	......	......	1
504,600	......	......	......	......	1	......	1	2
505,300	......	1	......	......	......	......	......	1
540,900	......	2	......	......	......	......	......	2
582,500	......	2	......	......	......	......	......	2
583,300	1	......	......	......	1	......	......	2
631,000	1	......	......	......	1	......	......	2
688,700	......	......	......	1	......	......	......	1
757,500	......	2	1	1	......	......	......	4
841,600	......	1	1	1	......	......	......	3
946,900	2	1	......	2	......	......	......	5
1,081,000	2	......	......	......	......	......	1	3
1,082,000	......	......	1	......	......	......	......	1
1,089,000	......	......	......	3	......	......	......	3
1,262,000	3	2	......	......	......	......	......	5
1,515,000	6	1	......	4	......	2	......	13
1,893,000	......	1	......	4	......	......	......	5
2,524,000	2	......	......	9	......	......	2	13
3,787,000	......	......	1	2	......	2	......	5
7,157,000	......	......	......	......	......	2	......	2
15,150,000	......	......	......	......	......	1	......	1
	17	15	7	27	8	7	5	86

[1] The certification of these lots was due to an unknown source of error in the analytical method; the analyses made at that time (July, 1909), showed less than 1 part of As_2O_3 per 400,000; see also page 204.

It is therefore somewhat premature to attempt to define rigidly the requirements for composition and purity for colors until a sufficient number of analyses is available to permit a hard-and-fast line to be drawn for each item as required for each color. Until that time the decision as to whether or not a certain color shall be certified must rest with the Department of Agriculture. However, the following requirements are tentatively suggested as being commercially practicable. It should be clearly understood that the tentative requirements here stated are based on the results of actual control, and are not any more searching or numerous than are the requirements for many if not most of the coal-tar dyes or their component parts in the industrial arts, particularly for the various kinds of paint, varnish, and ink making. While it may be that some of the tentative requirements herein defined necessitate the expenditure of considerable work and time, yet that is also true of some of the commercial requirements. Since manufacturers of cheap paints, varnishes, inks, and the like, find it wise and necessary carefully to control the quality of the coal-tar dyes or their component parts which they use, it can not be less wise or necessary to extend the same kind of quality control to those coal-tar dyes intended and sold for human consumption as food. This stand is fully justified by the quality of the coal-tar dyes offered as food colors on the United States markets as described in the foregoing pages. That such control is not only practical, but practicable, is fully proved by the fact that more than 20.5 tons (41,000 pounds) of coal-tar food dyes have been so controlled, examined, and certified under the food inspection decisions hereinbefore mentioned.

The figures are expressed in parts per hundred of actual color contained in the dye and not in parts per hundred of the total substance. The numbers at the head of the columns are the numbers in the Green Tables.

Tentative limits of composition suggested for permitted colors.

(Parts per hundred of actual color.)

Determinations.	Green Tables numbers.						
	4	56	85	107	435	517	692
Insolubles:							
Total	0.070	0.270	0.250	0.130	0.030	0.060	0.450
Nonvolatile on ignition	.040	.150	.050	.050	.010	.020	.250
Ether extractives:							
Neutral	.017	.090	.150	.030	.040	.035	.100
Alkaline	.004	.015	.035	.005	.006	.002	.030
Acid	.050	.017	.050	.010	.008	....	.150
Common salt	.600	5.000	2.000	2.500	.600	.400	3.000

In addition to these there are the following requirements applicable to all colors:

1. The absence of admixed dye must be convincingly demonstrated by suitable test.

2. *Arsenic.*—Test 17 of the United States Pharmacopœia, 1900, applied to so much of the specimen as represents 3.5 grams of actual dye must give a negative response. Such negative result must be reenforced by a check test identical with the test on the dye with the addition of 0.005 mg of arsenic (As_2O_3) to the dye prior to treatment, and this check test must produce a positive result for the presence of arsenic. Ignitions in the preparation of the material for the test must be made in porcelain.

Any other mode of testing which is demonstrated to be capable of detecting 0.005 mg added arsenic (As_2O_3) in so much of the specimen as represents 3.5 grams of actual color will, of course, be accepted. This quantity, however, is only tolerated tentatively pending further investigations relative to the complete, or practically complete, elimination of arsenic from foods, especially those which are largely used by children, such as candies.

3. *Heavy metals.*—Test 121 of the United States Pharmacopœia as revised May 1, 1907, using so much of the specimen as represents one part of actual dye must give a negative response for all metals except iron, which may be present in amounts not in excess of 0.005 per cent of iron based on the dye actually present in the specimen.

4. None of the dyes offered for certification shall contain any Glauber's salt or sodium sulphate in any form, nor shall they contain any added sugar, dextrin, or other loader, filler, or reducer for any purpose whatsoever, and convincing proof of the absence of any or all of them must be submitted.

5. The ether extractives are to be made successively upon water solutions of the dye, first neutral, then made alkaline with caustic soda, and then made acid with hydrochloric acid, using washed or sodium, dried ether.

6. The sulphur content of the sulphur-containing dyes must agree substantially with the theoretical; likewise the sulphated ash figures of all must agree substantially with the theoretical; variations between these two sets of figures, as long as they are consistent with each other, will not be reason for rejection.

7. In the case of No. 4 proof must be submitted showing that the specimen is of the sodium or potassium variety, and if it is a mixture of these two varieties the proportion of each present in the mixture must be stated.

8. In the case of No. 56 the crude cumidin employed may have a boiling point of from 220° to 230° C., and may be liquid or solid; the absence of any compound of S or G salt must be convincingly shown.

9. In the case of No. 85 convincing proof must be submitted that beta-naphthol orange if present at all is present in an amount not in excess of 5 per cent of the coal-tar dye present.

10. In the case of No. 107 the absence of any compound of S or G salt must be convincingly shown.

11. In the case of No. 435 the product should be free from calcium; convincing proof of absence of No. 434 must be submitted.

12. In the case of No. 517 the actual dye must contain not less than 56 per cent of iodin (sodium basis) and must not contain any other halogen; the kind and amount of metallic base, whether sodium, potassium, or the like, must be shown.

13. In the case of No. 692 the absence of indigo monosulphonic acid and of nonsulphonated indigo must be convincingly shown.

14. Each foundation certificate must be filed in duplicate, but need not be executed in duplicate and must contain a summarized or tabulated statement of all the quantitive results contained in the certificate, also a tabulation or summary of the qualitative tests made, together with the results of such tests, all stated on one sheet, so that the certificate will bear within itself its own summary and conclusions.

15. The fundamental analytical data must be given with such fullness as to permit efficient checking of the calculations, and the arithmetical operations performed should be indicated wherever needful to avoid confusion, or to facilitate the work of the checking chemist, or make the meaning of the certificate more plain.

There are freely quoted in the United States market two substances, paranitranilin and betanaphthol, which are subject to much competition, the prices for which, wholesale, are not far from 25 cents and 9 cents, respectively. A specimen of each has been examined, and, as before stated, they have both been found to be of such purity, with respect to arsenic, heavy metals, and general cleanliness, that had they been capable of use in food products, no objection against their use on this score could reasonably be raised; certainly no such objection could be successfully maintained.

The following table discloses the chemicals entering into the manufacture of paranitranilin and of betanaphthol, as well as of each of the seven permitted colors; the ingredients numbered 1 to 7 are used in the manufacture of these two substances, as well as in the seven permitted colors, as indicated by the "x" entries; ingredients 8 to 20 are used only in the manufacture of the seven permitted colors, and not in paranitranilin and betanaphthol.

Comparison of chemicals entering into the composition of the seven permitted colors and of paranitranilin and betanaphthol.

Determinations.	4. Napthol Yellow S.	56. Ponceau 3R.	85. Orange I.	107. Amaranth.	435. Light Green.	517. Erythrosin.	692. Indigo disulpho acid.	Paranitraniline.	Betanaphthol.
1. Naphthalene	x	x	x	x	....	x	x	....	x
2. Benzol	....	x	x	....	x	x	x	x	....
3. Sulphuric acid	x	x	x	x	x	x	x	x	x
4. Nitric acid	x	x	x	x	x	....	....	x	....
5. Metallic iron	....	x	x	x	x	....	....	x	....
6. Acetic acid	....	x	x	x	x	....	x	x	....
7. Caustic soda or potash	x	x	x	x	....	x	x	....	x
8. Carbonate of soda	x	x	x	x	x	....	x	....	....
9. Common salt	x	x	x	x	....	x	x	....	....
10. Wood or methyl alcohol	....	x	....	....	....	x	....	....	....
11. Lime	....	x	....	x	x	....	x	....	....
12. Hydrochloric acid	....	x	x	x	....	....	....	....	....
13. Sodium nitrite	....	x	x	x	....	....	....	....	....
14. Ethyl chlorid	....	....	....	....	x	....	....	....	....
15. Peroxid of lead or of manganese	....	....	....	....	x	....	....	....	....
16. Phosgene	....	....	....	....	x	....	....	....	....
17. Iodin	....	....	....	....	....	x	....	....	....
18. Metallic mercury	....	....	....	....	....	x	x	....	....
19. Ferric chlorid	....	....	....	....	....	x	....	....	....
20. Chlorin	....	....	....	....	....	....	x	....	....
	6	13	11	11	10	9	10	5	3

With respect to the arsenic content of the finished product, it can be asserted, without any fear whatever of successful contradiction, that the arsenic finds its way into the goods by way of the sulphuric acid which is used in the manufacture of all the permitted colors, as well as in the manufacture of Paranitranilin and Betanaphthol. If arsenic from this source can be kept out of these substances it can also be excluded from the permitted colors.

Turning now to those ingredients below the parallel lines, the only means of introducing arsenic would be through the hydrochloric acid used, which in turn derives its arsenic from the sulphuric acid used in its manufacture, and since arsenic free-sulphuric acid can be used in the first stages of producing the seven permitted colors and the paranitranilin and betanaphthol, it can also be employed in the manufacture of the hydrochloric acid used in the subsequent stages of manufacture of the seven permitted colors. Therefore, it seems unreasonable to permit a higher arsenic content in food colors than in paranitranilin and betanaphthol, which are sold in open competition, and which are used for the production of the very cheapest colored cloths, inks, and paints.

With reference to the content of iron, and other heavy metals, which satisfied the pharmacopœial tests in the case of paranitranilin and betanaphthol, these materials probably enter the product from the vessels in which the manufacturing operations are performed. The same kind of vessel used in the manufacture of paranitranilin and betanaphthol is used in making the seven permitted colors, and since the heavy metals can be and are kept out of these two substances to the extent required by the Pharmacopœia, there seems to be no good reason why they should not be kept out of the seven permitted colors when made for use in food products.

Since all these defects in the seven permitted colors can be obviated in their first stage of manufacture (as is shown in the case of paranitranilin and betanaphthol, and in some actual commercial samples of 1907, see sections XIV and XV), they are commercially avoidable at subsequent stages of manufacture, and there is no good reason why they should be then introduced.

XVII. METHODS OF ANALYSIS USED IN TESTING COLORS FOR CERTIFICATION.

INTRODUCTION.

The exact analytical methods developed and tested in the New York Food and Drug Inspection Laboratory and used in obtaining the analytical data contained in the preceding chapter are described in the following pages. These descriptions were written by A. F. Seeker, of that laboratory, under whose immediate supervision all the laboratory work was done, and they represent a great deal of work, extending over more than three years. The methods are submitted in the hope and expectation that in their wider application by a larger number of chemists any defects in the methods or conclusions drawn from the results will be detected and rectified. Experience in the New York laboratory has shown that even different chemists of varying degrees of experience in this particular line of work obtain concordant duplicates after a comparatively short laboratory acquaintance with these methods.

The identity of these colors and their freedom from foreign dyes is shown by the close agreement of their elements, as determined by analysis, with their theoretical composition; their behavoir toward reagents as given by standard works on dyes; their distribution between the layers when neutral, alkaline, and acid aqueous solutions are shaken with various immiscible solvents; uniformity of shade in the spots produced by particles of the dry color blown over the surface of wet filter paper, or water, and over concentrated sulphuric acid; uniformity of shade produced by a 0.5 per cent dyeing on wool under standard conditions, with similar dyeings from fractions produced by partial precipitation, by partial salting out, by fractional crystallization, and by extraction with alcohol or some liquid in which the pure color is not very soluble; and the behavior of the dye in acid and alkaline solution toward cotton. Which of these tests are needed to prove conclusively the identity of certain dyes or to establish their absence, is a matter that varies so much from case to case that it must be left largely to the individual judgment to decide on the best combination of tests, and for that reason such combinations are not here offered. The general methods and procedures just outlined have, however, when properly combined, led to satisfactory results.

In order to compare the results of the color analyses on the same basis, the actual figures obtained in the various determinations besides being reported as found, are also recalculated on a basis of 100 parts of actual color, i. e., the sum of the percentages of material which is not coloring matter, such as moisture, total insoluble matter, sodium chlorid, sodium sulphate, and ether extractives is deducted from 100 and the percentages of the various constituents found divided by the difference, the quotient being then multiplied by 100. The percentage of sodium in actual dye is calculated from the sulphated ash. The methods of analysis of the seven permitted colors of Food Inspection Decision No. 76 are given in the following order:

1. Naphthol Yellow S.
2. Ponceau 3R.
3. Orange I.
4. Amaranth.
5. Light Green S F Yellowish.
6. Erythrosin.
7. Indigo Disulphoacid.

NAPHTHOL YELLOW S.

MOISTURE.

Dry from 1 to 2 grams of the finely powdered dye in an air oven at 120° to 125° C. to constant weight.

TOTAL INSOLUBLE MATTER.

Dissolve 5 grams of color in 200 cc of hot distilled water, filter through a tared gooch, wash till the washings run through colorless, dry the insoluble residue at 105° C., and weigh.

NONVOLATILE OR INORGANIC INSOLUBLE MATTER.

Ignite the gooch containing the insoluble matter of the last determination at a low red heat, cool, and weigh.

SODIUM CHLORID.

Dissolve 3 to 5 grams of dye in 200 cc of water, acidify the solution with nitric acid, and precipitate the chlorin as silver chlorid. The latter is separated, washed, ignited, and weighed in a tared gooch crucible in the usual way.

SODIUM SULPHATE.

Dissolve 1 gram of dye in about 100 cc of water in a 200-cc graduated flask, add 40 cc of a 20 per cent solution of potassium chlorid, shake the mixture well, make up to mark with water, shake again, and then filter through dry paper. Treat 100 cc of the filtrate (representing 0.5 gram of dye) with 5 cc of 10 per cent barium chlorid solution without acidifying, and allow to stand overnight. If a precipitate forms, it is filtered, washed, ignited, and weighed in the usual way.

HEAVY METALS.

Treat the sulphated ash from 1 gram of the sample with 20 cc of water, digest with 10 cc of 10 per cent hydrochloric acid till solution is complete, place 3 cc of the mixture in a test tube, add 10 cc of freshly prepared hydrogen sulphid test solution (U. S. P.), shake the mixture, warm to 50° C., stopper, and allow to stand in a warm place (at about 35° C.) for half an hour. Run a blank test at the same time, with the same amount of hydrogen sulphid solution, using water instead of the solution containing the color ash. No turbidity other than that sometimes produced by slight separation of sulphur should appear in this test. Both tubes are then made slightly alkaline with ammonium hydroxid, and no precipitate should be produced, although a slight coloration, due to the presence of a small amount of iron, sometimes occurs. If this coloration is very marked the amount of iron should be determined. This is done by digesting the sulphated ash from a weighed amount of the sample with hydrochloric acid until all of the iron has gone into solution. The solution is filtered, and the filtrate poured into an excess of hot, pure, freshly prepared sodium hydroxid (by sodium) solution in a platinum dish. The precipitate is washed, dissolved in dilute hydrochloric acid, and again precipitated with ammonium hydroxid. The last precipitate is washed, ignited, and weighed in the usual manner.

ARSENIC (SEEKER AND SMITH'S METHOD).

Dissolve 10 grams of the dye in 200 cc of water, heating to insure complete solution of the color, add about 10 cc of strong bromin water to convert any arsenite to arsenate. Make the mixture alkaline with a few cubic centimeters of strong ammonium hydroxid. Twenty cubic centimeters of a sodium phosphate solution containing 100 grams of crystallized sodium phosphate per liter are next added from a pipette, after which magnesia mixture (containing 55 grams of hydrated magnesium chlorid, 55 grams of ammonium chlorid, and 88 cc of ammonium hydroxid, specific gravity 0.9 per liter) is added from a burette, stirring vigorously. The amount of magnesia added should be in slight excess of that necessary completely to precipitate the phosphate and should previously be ascertained by blank experiment. Then add 10 cc of ammonium hydroxid (specific gravity 0.90), and allow the whole to stand for at least three hours; separate the precipitate by filtration and wash it free, or nearly so, of dye with ammonium hydroxid containing one-tenth its volume of ammonia (specific gravity 0.90). Dissolve the precipitate from the paper with 1:1 nitric acid, the washings being collected in a large porcelain crucible; add 5 cc of sulphuric acid to the contents of the crucible, and evaporate the whole almost to dryness. It is not necessary that the solution should be colorless at this point, a brown colored solution giving equally accurate results. Add 20 cc of water to the residue in the crucible and then 10 cc of a saturated solution of sulphur dioxid; evaporate the solution to a sirupy consistency to remove the sulphur dioxid, and then take up in 20 cc of water and place in a 30-cc evolution bottle, add 5 cc of concentrated sulphuric acid, and determine the arsenic in the form of apparatus (fig. 1) used by Bishop in his modification of the Gutzeit test, the stains obtained being compared with those given by known amounts of arsenic.

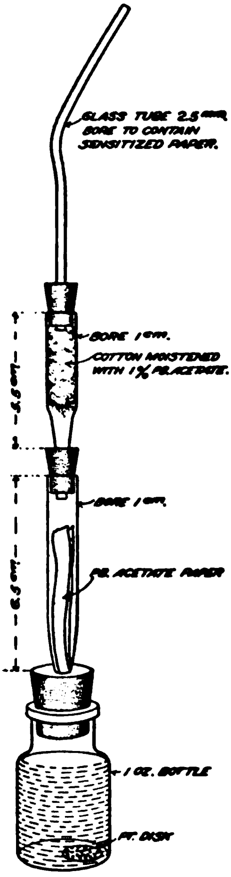
GLASS TUBE 2.5 mm BORE TO CONTAIN SENSITIZED PAPER.
BORE 1 cm.
COTTON MOISTENED WITH 1% PB. ACETATE.
5.5 cm
BORE 1 cm.
PB. ACETATE PAPER
6.5 cm
1 OZ. BOTTLE
PT. DISK

FIG. 1.—Apparatus for the determination of arsenic.

The apparatus used by Bishop consists of the following parts: A 30-cc salt mouth evolution bottle into which is fitted a one-hole rubber stopper carrying a glass tube 6.5 cm long with an internal diameter of 1 cm, this tube in turn being provided with a one-hole rubber stopper fitted with another tube of the same diameter and 5.5 cm long, the diameters of both tubes being constricted at the points at which they are inserted in the rubber stoppers. A third glass tube 15 cm long, having an internal diameter of 2.5 mm, is fitted into the second at its upper end by means of a rubber stopper. The first tube contains a strip of filter paper which has been saturated with a 5 per cent solution of lead acetate and dried. The second tube contains a loosely packed plug of cotton-wool freshly moistened with a 1 per cent solution of lead acetate. Into the topmost tube is inserted the strip of sensitized paper to receive the arsenic stain. The arsin is generated by introducing into the evolution bottle six pieces of Kahlbaum's stick zinc (arsenic-free for forensic purposes) weighing in all about 8 grams, and to assist in an active and constant evolution of gas a disk of platinum is also placed in each bottle to form an electrolytic couple. The evolution of gas is allowed to proceed for one hour. The stains are produced on strips of hard pressed white paper (2 mm wide and 120 mm long) that has been sensitized by being dipped in a 5 per cent alcoholic solution of mercuric chlorid and then dried. (NOTE.—It has recently been found that mercuric bromid yields stains that are more evenly distributed and also produces standards that are incomparably more permanent.) For purposes of comparison it is better not to develop the strips stained by the arsin, though some prefer to dip the stains in ammonium hydroxid, which causes them to become black. A blank is run with each set of determinations.

ETHER EXTRACTIVES.

Dissolve 10 grams of color in 150 cc of water and extract in a separatory funnel with ether that has been washed with water (using three 150 cc portions of water for each liter of ether). Extract the color solution with two 100 cc portions of this ether, shaking thoroughly for one minute, and wash the combined ether extract successively with 35, 20, and 10 cc of water made alkaline or acid, as the case requires, with 1 cc of tenth normal alkali or acid per 100 cc of water. Decant the ether from the mouth of the separatory and rinse the funnel once with 5 cc ether. The color solution is first extracted neutral, the extracted solution being then rendered alkaline with 2 cc of a 10 per cent solution of caustic soda and again extracted with two 100-cc portions of ether. In acidifying for the third extraction, add twice the amount of hydrochloric acid (1 to 3) necessary to neutralize the alkali, and repeat the extraction with two 100-cc portions of ether. Place the neutral, alkaline, and acid extracts in a dust-free atmosphere, and allow the ether to evaporate spontaneously, after which dry the residues to constant weigh over sulphuric acid, using flat-bottomed dishes 2¾ inches in diameter, 1¼ inches in height, and of about 100 cc capacity. The dishes should be thoroughly cleaned, wiped dry, and allowed to stand in a sulphuric acid desiccator at least two hours before weighing. In order to avoid the generation of static charges of electricity, they should not be wiped immediately before weighing. Run two blank determinations with each series of ether extracts, and deduct the average gain in weight of these two blanks from the weights obtained in the other determinations.

SULPHATED ASH.

Weigh accurately 0.5 to 1 gram of the color into a wide platinum dish, moisten with concentrated sulphuric acid, and ignite cautiously, avoiding spattering; moisten the residue repeatedly with sulphuric acid and ignite until all the carbon is removed and a white or reddish ash is obtained. This is finally ignited at a fairly bright red heat, cooled, and weighed. The aqueous solution of this ash should be neutral to litmus, and may be used in a quantitative test for potassium.

CALCIUM.

Digest the residue from the sulphated ash determination with hydrochloric acid, render the solution alkaline with ammonium hydroxid, filter, and precipitate the calcium in the filtrate with ammonium oxalate. The precipitate of calcium oxalate is filtered on a tared gooch, washed, dried at 100° C. and weighed as calcium oxalate, the calcium being calculated from the formula $CaC_2O_4.H_2O$. This method is applicable only in cases where the amount of calcium is very small. When the amount exceeds 0.5 per cent it should be determined by digesting the sulphated ash with ammonium sulphate solution made acid with hydrochloric acid, and precipitating the iron by an excess of ammonium hydroxid. The precipitate is washed, dissolved in hydrochloric acid, and reprecipitated, the filtrate and washings being added to those obtained from the first precipitate. The calcium is precipitated in the combined filtrates with ammonium oxalate, the oxalate being filtered, washed, ignited, and weighed in the usual way; the residue is weighed as oxid after ignition to bright redness.

SULPHUR.

Determine upon 0.2 to 0.3 gram portions by the Carius method (Gattermann, Practical Methods of Organic Chemistry, 1901, p. 81), using 3 cc of fuming nitric acid (sp. gr. 1.5), and heating the sealed tubes to 300° C. for at least eight hours.

NITROGEN.

Use the method of Dumas (Gattermann, Practical Methods of Organic Chemistry, 1901, p. 85).

PONCEAU 3R.

MOISTURE.

Dry 1 to 2 grams of the finely ground dye at 109° to 110° C. in a current of dry hydrogen to constant weight.

TOTAL INSOLUBLE MATTER.

Determine as under Naphthol Yellow S, page 211.

NONVOLATILE OR INORGANIC INSOLUBLE MATTER.

Determine as under Naphthol Yellow S, page 211.

SODIUM CHLORID (SEEKER AND MATHEWSON'S METHOD).

Mix 2 grams of dye thoroughly with from 2 to 3 grams of sodium carbonate, moisten with water to form a paste, again mix, dry, and ignite at a low red heat. By moistening, drying, and reigniting a more complete destruction of organic matter is obtained. Break up the charred mass, introduce into a 200-cc graduated flask with about 100 to 150 cc of hot water, and add an excess of potassium permanganate to oxidize sulphids. Destroy the excess of permanganate by adding sulphur dioxid solution until the red color changes to brown, then cool the mixture and make up to the mark with water. Filter through a dry paper, acidify 100 cc of the filtrate with nitric acid, and precipitate the chlorin as silver chlorid by the addition of silver nitrate. If the solution should be brownish from a trace of organic matter the silver chlorid does not readily coagulate and tends to pass through the filter. In this case a few drops of potassium permanganate solution are added to this acid mixture, the organic matter being almost instantly oxidized. The mixture is then decolorized with a few drops of sulphur dioxid solution, the silver chlorid is separated on a tared gooch, washed, ignited, and weighed in the usual manner.

The following method may also be used for Ponceau 3R:

Dissolve 5 grams of the dye in 15C cc hot water, wash into a 250-cc graduated flask, and add 25 cc of a 10 per cent solution of barium nitrate. Cool the mixture, make up to the mark, and filter through a dry paper; acidify 100 cc of the filtrate, representing 2 grams of color, with nitric acid, and treat with silver nitrate solution, the precipitated silver chlorid being separated, washed, ignited, and weighed in a tared gooch crucible in the usual way.

SODIUM SULPHATE.

Dissolve 2 grams of dye in 100 cc of hot water, wash into a 200-cc graduated flask, add 50 grams of pure sodium chlorid, cool, and make up to the mark with water. Filter through a dry filter, dilute 100 cc of the filtrate to 300 cc, acidify with hydrochloric acid and precipitate the sulphates with barium chlorid. Filter, wash, ignite, and weigh in a tared platinum gooch in the usual way.

HEAVY METALS.

Determine as given under Naphthol Yellow S, page 212.

ARSENIC.

Determine as given under Naphthol Yellow S, page 212.

ETHER EXTRACTIVES.

Determine as given under Naphthol Yellow S, page 214.

SULPHATED ASH.

Determine as given under Naphthol Yellow S, page 214.

CALCIUM.

Determine as given under Naphthol Yellow S, page 215.

SULPHUR.

Determine as given under Naphthol Yellow S, page 215.

NITROGEN (SEEKER AND MATHEWSON'S METHOD).

Treat 2 grams of the color with 25 cc of a saturated solution of sulphur dioxid and 1 gram of zinc dust and warm the mixture gently until it becomes colorless. This should take place in from two to three minutes, but if it does not add more sulphur dioxid solution in small portions at a time until the color is destroyed. Then add 30 cc of concentrated sulphuric acid and 0.7 gram of mercuric oxid or its equivalent of metallic mercury and digest the mixture, make alkaline, and distil as directed on page 6, Bulletin 107, Revised, Bureau of Chemistry, United States Department of Agriculture, under the Kjeldahl process.

CRUDE CUMIDIN.

Dissolve 20 grams of dye in 400 cc of hot water and pour the solution, a little at a time, into a reducing solution composed of 75 grams of stannous chlorid dissolved in 180 cc of concentrated hydrochloric acid. Heat the mixture on a steam bath until it is straw colored, cool, add an excess of sodium hydroxid, and extract in a separatory funnel with ether. Separate the ether layer and distil off the solvent until the residue measures about 50 cc. Then cautiously heat over the steam with constant agitation until the odor of ether disappears, after which the last of the moisture is removed by introducing a few pieces of solid caustic soda and allowing to stand. The residue consists of crude cumidin and should boil above 220° C. Cumidin nitrate is sparingly soluble in water.

ORANGE I.

MOISTURE.

Determine as given under Ponceau 3R, page 215.

TOTAL INSOLUBLE MATTER

Determine as given under Naphthol Yellow S, page 211.

NONVOLATILE OR INORGANIC INSOLUBLE MATTER.

Determine as given under Naphthol Yellow S, page 211.

SODIUM CHLORID.

Determine as given under Ponceau 3R, page 215.

SODIUM SULPHATE.

Dissolve 1 gram of dye in 100 cc water contained in a 200-cc graduated flask and treat the solution with 60 cc of a 20 per cent solution of potassium chlorid. Make the mixture up to the mark with water, shake, filter through a dry paper, dilute an aliquot of 100 cc of the filtrate to 200 cc, acidify with 1 cc of 10 per cent hydrochloric acid, treat with 5 cc of 10 per cent barium chlorid solution, and allow to stand over night. If a precipitate has been formed this is separated, ignited, and weighed in the usual way.

HEAVY METALS.

Determine as given under Naphthol Yellow S, page 212.

ARSENIC (SEEKER AND SMITH'S METHOD).

Dissolve 2 grams of dye in a mixture of 130 cc water and 70 cc of 95 per cent alcohol. Add about 10 cc of strong bromin water to convert any arsenite to arsenate. Make the mixture alkaline with a few cubic centimeters of strong ammonium hydroxid and add, from a pipette, 20 cc of a sodium phosphate solution containing 100 grams of crystallized sodium phosphate per liter, after which magnesia mixture (containing 55 grams of hydrated magnesium chlorid, 55 grams of ammonium chlorid, and 88 cc of ammonium hydroxid, sp. gr. 0.9, per liter) is added from a burette, stirring vigorously. The amount of magnesia mixture to be added should be in slight excess of that necessary to precipitate the phosphate completely, and should be previously ascertained by a blank experiment. Finally add 10 cc of ammonium hydroxid (sp. gr. 0.96) and allow the whole to stand for at least eight hours. Separate the precipitate by filtration and wash it free, or nearly so, of dye with a mixture of one-third alcohol and two-thirds water containing one-tenth its volume of ammonium hydroxid (sp. gr. 0.90). Dissolve the precipitate from the paper with 20 per cent sulphuric acid, the washings being collected in a large porcelain crucible. Add 5 cc of concentrated nitric acid to the contents of the crucible and evaporate the whole almost to dryness. The mixture need not be colorless at this point, a brown colored solution giving equally accurate results. Add 20 cc of water to the residue in the crucible and then 10 cc of a saturated solution of sulphur dioxid. Evaporate the solution to a syrupy consistency to remove sulphur dioxid, and then take up in 20 cc of water, place in a 30-cc evolution bottle, add 5 cc of concentrated sulphuric acid, and determine the arsenic by the modified Gutzeit method as given under Naphthol Yellow S, page 213.

ETHER EXTRACTIVES.

Determine as given under Naphthol Yellow S, page 214

SULPHATED ASH.

Determine as given under Naphthol Yellow S, page 214.

CALCIUM.

Determine as given under Naphthol Yellow S, page 215.

SULPHUR.

Determine as given under Naphthol Yellow S, page 215.

NITROGEN.

Determine as given under Ponceau 3R, page 217.

TEST FOR ORANGE II (SMITH AND MATHEWSON'S METHOD).

The following solutions are required:

(1) Fifteen per cent titanium trichlorid.

(2) Freshly prepared diazotized sulphanilic acid. Heat a mixture composed of 1 gram of sulphanilic acid, 10 cc of water, and 20 cc of concentrated hydrochloric acid on a steam bath for five minutes with occasional shaking. Cool to about 10° C. and add slowly 10 cc of a 1 per cent solution of sodium nitrite. Allow the mixture to stand at about 10° for 10 minutes, dilute to 1 liter, and shake until all the solid particles have dissolved.

(3) Stannous chlorid, prepared by dissolving 40 grams of stannous chlorid in 100 cc of concentrated hydrochloric acid. Dilute this ten times with water, immediately before using.

Place 2 cc of a 0.1 per cent solution of the dye in a colorimeter tube having a capacity of about 100 cc, add a small drop of titanium trichlorid solution and shake until the mixture is decolorized. Standards containing the same quantity of color composed of a mixture of pure Orange I and known amounts of Orange II are treated in the same way, the volume of the solution at this point to measure less than 5 cc. Dilute the decolorized solutions to 50 cc with 95 per cent alcohol and equalize the temperature by immersing the tubes in water at room temperature. (Note.—A slight coloration that may develop at this point may be disregarded.) Allow the tubes to stand in the water for about five minutes, add 2 cc of the diazotized sulphanilic acid, and mix thoroughly. If the titanium trichlorid has not been used in too great excess, the first few drops of the diazotized sulphanilic acid will cause a formation of color. Allow the coupling to proceed for three minutes and then add 5 cc of the diluted stannous chlorid with vigorous shaking. In two minutes the blue color due to Orange I will disappear, leaving only the pink caused by the coupling product of Orange II. The depth of color in the tube containing the dye under examination may then be compared to the standards.

AMARANTH.

MOISTURE.

Determine as given under Ponceau 3R, page 215.

TOTAL INSOLUBLE MATTER.

Determine as given under Naphthol Yellow S, page 211.

NONVOLATILE OR INORGANIC INSOLUBLE MATTER.

Determine as given under Naphthol Yellow S, page 211.

SODIUM CHLORID.

Determine as given under Ponceau 3R, page 215.

SODIUM SULPHATE.

Dissolve 2 grams of dye in 100 cc of warm water in a 200-cc graduated flask, and add 36 grams of pure sodium chlorid. Allow the mixture to stand with frequent shaking for one hour, and after cooling make up to the mark with a saturated salt solution. Shake the mixture and filter through a dry paper; dilute 100 cc of the filtrate (representing 1 gram) with water, acidify with hydrochloric acid, and precipitate the sulphates with barium chlorid. The precipitate is separated, washed, and ignited upon a tared platinum gooch crucible.

HEAVY METALS.

Determine as given under Naphthol Yellow S, page 212.

ARSENIC.

Determine as given under Naphthol Yellow S, page 212.

ETHER EXTRACTIVES.

Determine as given under Naphthol Yellow S, page 214.

SULPHATED ASH.

Determine as given under Naphthol Yellow S, page 214.

CALCIUM.

Determine as given under Naphthol Yellow S, page 215.

SULPHUR.

Determine as given under Naphthol Yellow S, page 215.

NITROGEN.

Determine as given under Ponceau 3R, page 217.

LIGHT GREEN S F YELLOWISH.

MOISTURE.

Determine as given under Ponceau 3R, page 215.

TOTAL INSOLUBLE MATTER.

Determine as given under Naphthol Yellow S, page 211.

NONVOLATILE OR INORGANIC INSOLUBLE MATTER.

Determine as given under Naphthol Yellow S, page 211.

SODIUM CHLORID.

Determine as given under Ponceau 3R, page 215.

SODIUM SULPHATE.

Dissolve 5 grams of the color in 100 cc of water, warming by means of a gentle heat. Dissolve 11 grams of safranin in a separate portion of 400 cc of water, also by warming, taking care in both instances to prevent loss by evaporation. Mix the two solutions, shake thoroughly, and filter through a dry filter. Render an aliquot portion of the filtrate alkaline with sodium hydroxid and remove the excess of safranin by shaking with two successive portions of amyl alcohol. Wash the combined amyl alcohol layers with two portions of water and add the washings to the main aqueous solution, which is then acidified with hydrochloric acid and sulphates determined in the usual manner by preciptiation with barium chlorid as barium sulphate.

HEAVY METALS.

Determine as given under Naphthol Yellow S, page 212.

ARSENIC.

Determine as given under Naphthol Yellow S, page 212.

ETHER EXTRACTIVES.

Determine as given under Naphthol Yellow S, page 214.

SULPHATED ASH.

Determine as given under Naphthol Yellow S, page 214.

CALCIUM.

Determine as given under Naphthol Yellow S, page 215

SULPHUR.

Determine as given under Naphthol Yellow S, page 215.

NITROGEN.

Determine on 2-gram portions by Gunning's modification of the Kjeldahl process, using a little copper sulphate to assist the oxidation (see page 7, Bulletin 107, Revised, Bureau of Chemistry, United States Department of Agriculture).

ERYTHROSIN.

MOISTURE.

Determine as given under Ponceau 3R, page 215.

TOTAL INSOLUBLE MATTER.

Determine as given under Naphthol Yellow S, page 211.

NONVOLATILE OR INORGANIC INSOLUBLE MATTER

Determine as given under Naphthol Yellow S, page 211.

SODIUM CHLORID.

Dissolve 5 grams of the dye in 400 cc water and acidify with dilute nitric acid. Make the mixture up to 500 cc with water, and then filter through a dry filter. Determine chlorids in an aliquot of 200 cc of the filtrate by precipitation with silver nitrate, washing, igniting, and weighing the silver chlorid in a tared gooch crucible in the usual manner.

SODIUM SULPHATE.

Employ another aliquot of the filtrate obtained after precipitating the color acid as above in the determination of sulphates, precipitating the latter as barium sulphate in the usual manner.

HEAVY METALS.

Determine as given under Naphthol Yellow S, page 212.

ARSENIC (SEEKER AND SMITH'S METHOD).

Dissolve 16 grams of dye in 370 cc of water, add 5 cc of strong bromin water, and finally 25 cc of dilute sulphuric acid (1 to 4). Shake thoroughly and filter through a dry filter. Place an aliquot of 250 cc, representing 10 grams of color from the filtrate, in a porcelain casserole, add 5 cc concentrated nitric acid (very important to prevent loss of arsenic), and evaporate till fuming has ceased. Reduce the residue with sulphur dioxid solution, evaporate to small bulk, and determine the arsenic in the form of apparatus used by Bishop in his modification of the Gutzeit test. See under Naphthol Yellow S, pages 212 and 213. (NOTE.—It is somewhat difficult at times to recover 250 cc of filtrate, but less may be used and a correction made, if necessary.)

ETHER EXTRACTIVES.

Determine as given under Naphthol Yellow S, page 214, omitting the acid extraction.

SULPHATED ASH.

Determine as given under Naphthol Yellow S, page 214.

IODIN, BROMIN, AND CHLORIN (CORNELISON'S METHOD).

Mix 0.2 to 0.3 gram of the sample with 2 grams of pure potassium bichromate and 15 cc of strong sulphuric acid in the evolution flask of an apparatus made entirely of glass, with ground-glass joints. Thoroughly mix the contents of the evolution flask, so that all lumps are disintegrated, and then heat at 100° C. for 15 minutes, after which raise the temperature to 150° C. for thirty minutes, a current of air dried over calcium chlorid and potassium hydroxid being drawn through the apparatus during this time. Iodin remains in the evolution flask as iodic acid; bromin passes off as such, and may be absorbed by allowing the air passing through the apparatus to bubble through 1 per cent sodium hydroxid. Chlorin passes out of the evolution flask as chromyl chlorid, and may also be absorbed in sodium hydroxid. Cool the mixture containing the iodic acid, and reduce the chromic acid by addition of sulphur dioxid, about 20 cc of a saturated solution being required. When enough has been added, the precipitated iodin redissolves, and the clear green color of chrome alum appears. Filter, wash the paper with distilled water, dilute the filtrate and washings to about 300 cc, and add an excess of silver nitrate. Boil till the silver iodid has flocculated, allow to stand for a few hours, and separate and weigh the silver iodid in a tared gooch.

It sometimes happens that the mixture containing the iodic acid, after the reduction with sulphur dioxid, becomes turbid, owing, apparently, to separation of a basic chromium sulphate. Very often the turbidity can not be removed by filtering, and it has been found advisable in this case to reject the determination and begin anew.

IODIN (SEEKER AND MATHEWSON'S METHOD).[1]

Place from 0.3 to 0.4 gram of the erythrosin in a porcelain casserol, dissolve this in 5 cc of a 10 per cent sodium hydroxid solution, then add 35 cc of a 7 per cent solution of potassium permanganate. After mixing, cover the vessel with a watch crystal, and add 10 cc of nitric acid, keeping the dish covered. Agitate the mixture, place on a steam bath, and keep covered until spattering ceases, after which remove the watch glass and allow evaporation to proceed to dryness.[1] Treat the residue with 5 cc of 7 per cent potassium permanganate and 5 cc of concentrated nitric acid and again evaporate to dryness. Then add about 50 cc of water and 5 cc of concentrated nitric acid to the residue, following this by 40 cc of a saturated solution of sulphur dioxid, and allow the whole to stand with occasional stirring (breaking up the lumps with a glass rod) until the hydrated oxid of manganese has dissolved. Filter, and wash the paper with water. Add an excess of silver nitrate to the filtrate, and boil until sulphur dioxid has been expelled and the silver iodid has flocculated. Separate, wash, and weigh the precipitate in the usual manner.

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[1] U. S. Dept. Agr., Bureau of Chemistry Circular 65. The estimation of iodin in organic compounds and its separation from other halogens.

TOTAL HALOGENS.

Mix 0.5 to 1 gram of the dye with 4 grams of potassium carbonate, moisten to a paste, again thoroughly mix, cover with a layer of dry potassium carbonate, dry, and ignite at a low red heat. Break up the char thoroughly, digest with about 200 cc water, and filter. Wash the insoluble matter until the washings no longer react with silver nitrate; then acidify the filter and washings with nitric acid and precipitate the halogens in the usual way as silver salts.

INDIGO DISULPHOACID.

MOISTURE.

Determine as given under Ponceau 3R, page 215.

TOTAL INSOLUBLE MATTER.

Determine as given under Naphthol Yellow S, page 211.

NONVOLATILE OR INORGANIC INSOLUBLE MATTER.

Determine as given under Naphthol Yellow S, page 211.

SODIUM CHLORID.

Determine as given under Ponceau 3R, page 215.

SODIUM SULPHATE.

Dissolve 2 grams of the dye in about 160 cc of water and treat the solution with 40 grams of pure sodium chlorid. After the salt has dissolved, make up the volume to exactly 200 cc. Shake the mixture thoroughly and filter through a dry filter. Dilute 50 cc of the filtrate with 200 cc of water, acidify with 1 cc of 10 per cent hydrochloric acid, and treat with an excess of barium chlorid solution. After standing overnight, the precipitate is separated, washed, ignited, and weighed in the usual way.

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[1] In the operation of drying particular care should be observed to prevent access of reducing gases to the mixture.

HEAVY METALS.

Determine as given under Naphthol Yellow S, page 212.

ARSENIC (SEEKER AND SMITH'S METHOD).

Treat 10 grams of dye in a Kjeldahl flask with 100 cc water and 10 cc concentrated nitric acid. Warm gently and finally boil until all action has ceased. Transfer to a beaker, make alkaline with ammonium hydroxid, and proceed from this point, as in the case of the solution of Naphthol Yellow S, page 212, continuing from the point at which sodium phosphate is added.

ETHER EXTRACTIVES.

Dissolve 3 grams of dye in 200 cc of water and extract with ether, as directed under Naphthol Yellow S, page 214.

SULPHATED ASH.

Determine as given under Naphthol Yellow S, page 214.

CALCIUM.

Determine as given under Naphthol Yellow S, page 215.

SULPHUR.

Determine as given under Naphthol Yellow S, page 215.

NITROGEN.

Determine as given under Light Green SF Yellowish, page 222.

XVIII. ADDENDA.

ADDITIONAL EXAMINATION OF COAL-TAR DYES.

Since the foregoing report was written the chemical examination of the coal-tar dye specimens collected in the summer of 1907 has been completed. This investigation shows that 7 of the Green Table numbers then reported were not on the market, although those furnishing the specimens included them at the time that the samples were supplied. These numbers are as follows: *not physiologically examined,*

G. T. 49, 60, 104, 518, 523; *physiologically examined,* with unfavorable result, G. T. 516; with favorable result, G. T. 520.

The chemical examination also shows that each of 9 additional Green Table numbers occurred once among the unreported numbers. They are as follows: *not examined physiologically,* G. T. 20, 328; *examined physiologically:* with unfavorable results, G. T. 602; with favorable results, G. T. 92, 477, 521; with contradictory results, G. T. 16, 43, 163. G. T. 20 is anilin azo (1:8) dioxy naphthalene (3:6) disulpho acid. G. T. 328 is diamino-stilbene-disulpho acid-disazo-phenol.

This chemical examination finally shows that beside the Green Table numbers there occurred, once each, the following: sulphonated Victoria Blue B and a sulphonated rhodulin and twice, amido-azo-toluene-azo-alpha-naphthol.

It is clear that this revised statement of facts is without influence on the 7 permitted dyes selected; its only effect is to add G. T. 92 (yellow), 477 (blue), and 521 (red) (each occurring but once) to, and to subtract 520 (red) (said to occur twice) from, the 16 dyes (page 166) from among which the 7 permitted dyes were selected, thus giving 18 instead of 16 dyes to choose from; however, the final selection is wholly unaffected thereby. It is also clear that any and all intermediate conclusions or comparisons are only slightly and immaterially affected, if at all, by the results of this additional examination.

SUPPLEMENTARY LIST OF TRADE NAMES OF COAL-TAR COLORS.[1]

Acid Yellow S (4).
Alkali Blue (477).
Azo Acid Rubin 2B (107).
Azo Yellow (92).
Crocein Scarlet O extra (164).
Dimethylanilin Orange (87).
Eosin, water soluble (512).
Fast Red A (102).
Fast Red B (65).
Fast Red E (105).
Fast Yellow Y (8).
Indian Yellow (92).
Indulin R and B (601).
Leucindophenol (572).
Methyl Orange (87).
Naphthylamin Pink (614).
Naphthylene Blue R in crystals (639).
Soluble Blue 8B and 10B (479).
Sulphur Yellow (4).
Thiochromogen (659).
Victoria Blue (488).
Wool Black (166).
Yellow T (84).

XIX. INDEX OF AUTHORITIES QUOTED.

[References are made to important citations exclusive of pages 76 to 152, except in cases where the author is not cited elsewhere.]

Page.
Arloing and Cazeneuve........... 90
Beckh........................... 55
Béhal........................... 177
Bergeron and Clouet............. 119
Beythien and Hempel............. 173
Bishop.......................... 213
Bokorny......................... 54
Braunschweig and Graefe......... 121
Buss............................ 71
Cazeneuve....................... 93
Cazeneuve and Arloing........... 90
Cazeneuve and Lépine............ 42
Chlopin................... 59, 72, 75, 181
Clouet and Bergeron............. 119
Combemale and François.......... 142
Confectioners' List............. 74
Dietrich........................ 37
Erdmann......................... 44, 57
Ernst........................... 170
Fraenkel, J..................... 25
Fraenkel, S.......... 60, 74, 160, 168, 180
François and Combemale.......... 142
Frentzel........................ 26
Galliard........................ 143
Gattermann...................... 215
Georgievics..................... 58
Graefe and Braunschweig......... 121

Page.
Green, Arthur G................. 15
Gudeman......................... 24
Heidenhain...................... 52
Hempel & Beythien............... 173
Henley.......................... 25
Houghton........................ 55
Hueppe.......................... 34
International White Cross Congress.......................... 172
Kayser.......................... 44
Kobert.......................... 83
Koenig.......................... 60
Kohnstamm, E. G................ 190
Lehmann......................... 57, 157
Lépine & Cazeneuve.............. 42
Lewin........................... 58
Lieber.......................... 74
Ludwig.......................... 24, 191
Meyer........................... 24, 60
Muttelet........................ 171
National Confectioners' Association........................ 30, 45
Neufeld, C. A................... 14
Patents, H. R. Committee on..... 61
Penzoldt........................ 55
Pfeffer......................... 51, 180
Possetto........................ 26

---

[1] See also page 148.

Page.
Rosenstiehl........................ 54
Santori........................... 47, 158
Schacherl..................... 45, 75, 191
Schultz.......................... 56
Society of Swiss Analytical Chemists.......................... 43, 75
Stilling..................... 55, 56, 57, 180
Canton of Tessin................. 75
Tschirch......................... 43, 57

Page.
v. Raumer........................ 37
Weyl............... 23, 24, 26, 44, 58, 181
Weyl (Leffman translation)....... 14, 37, 74, 179, 181
Williams, H. W................... 55
Winogradow...................... 52
Winton.......................... 24, 59
Young........................... 159

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U. S. DEPARTMENT OF AGRICULTURE,

BUREAU OF CHEMISTRY—BULLETIN No. 148.

H. W. WILEY, CHIEF OF BUREAU.

THE TOXICITY OF CAFFEIN:

AN EXPERIMENTAL STUDY

ON DIFFERENT SPECIES OF ANIMALS.

BY

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AND

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WASHINGTON:
GOVERNMENT PRINTING OFFICE.
1912.

LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Chemistry,
Washington, D. C., November 14, 1911.

Sir: I have the honor to submit for your approval a manuscript on the toxicity of caffein, which is the first of a series of reports to be made by Dr. Salant on the pharmacology of this drug; the conclusions here reported are, therefore, in some particulars to be regarded as tentative. The data obtained are primarily of use in the execution of the food and drugs act, but are capable of much broader application.

Acknowledgment is made of the assistance rendered by Dr. John R. Mohler, Chief of the Pathological Division, Bureau of Animal Industry, and his assistants, in performing the autopsies recorded in this report. I recommend the publication of the manuscript as Bulletin No. 148 of the Bureau of Chemistry.

Respectfully,

H. W. Wiley, *Chief.*

Hon. James Wilson,
Secretary of Agriculture.

THIS PUBLICATION may be procured from the Superintendent of Documents, Government Printing Office Washington, D. C., at 15 cents per copy

CONTENTS.

Page.

Introduction.......... 5
Historical review of the literature on the toxicity of caffein.......... 9
Acute caffein intoxication.......... 18
    Experiments on rabbits.......... 18
        Subcutaneous injection.......... 18
        Administration by mouth.......... 26
        Injection into the peritoneal cavity.......... 28
        Intramuscular injection.......... 33
        Intravenous injection.......... 37
        Summary.......... 42
    Experiments on guinea pigs.......... 43
        Subcutaneous injection.......... 43
        Injection into the peritoneal cavity.......... 47
        Administration by mouth.......... 49
        Summary.......... 52
    Experiments on cats.......... 53
        Subcutaneous injection.......... 53
        Injection into the peritoneal cavity.......... 56
        Administration by mouth.......... 57
        Summary.......... 59
    Experiments on dogs.......... 60
        Administration by mouth.......... 60
        Subcutaneous injection.......... 60
        Experiments on puppies.......... 61
        Summary.......... 62
Chronic caffein intoxication.......... 63
    Experiments on rabbits.......... 63
    Experiments on dogs.......... 75
Discussion of results.......... 91
General summary and conclusions.......... 95
Bibliography.......... 97

THE TOXICITY OF CAFFEIN.

INTRODUCTION.

Comparative physiology has established the fundamental fact that some properties are common to all forms of living matter. But the same method of inquiry has also led to the recognition of marked differences in the physiological processes of various species of animals. Among the most important investigations which contributed to the knowledge of such variation of function are the studies in comparative metabolism. It is now recognized that metabolism is in some respects quite different in herbivora and in carnivora. Some forms of oxidation are much greater in the rabbit than in cats and dogs. Nuclein metabolism presents important differences in the rabbit and in man, while the mode of neutralizing acid in the body may be cited as another variation in the metabolism of these forms. Perhaps the most striking examples of differences in the metabolism of different organisms is furnished by the results of studies on the fate of certain poisons introduced into the body.

The classical experiments of Bunge and Schmiedeberg[15] [a] on the synthesis of hippuric acid are of interest in this regard. It will be recalled that in the dog the synthesis takes place in the kidney; the rabbit is able to form hippuric acid in the liver as well as in the kidney, while frogs can synthesize hippuric acid even when both of these organs have been removed or excluded from the circulation. Observations on the fate of some of the alcohols of the fatty acid series have likewise shown that these substances may be combined with glycuronic acid in some animals but not in others. According to Thierfelder and Von Mering,[84] tertiary alcohols are combined in this manner in the rabbit but not in the dog. According to Neubauer,[64] the primary and secondary alcohols are so combined in the dog as well as in the rabbit, but to a greater degree in the latter.

Pohl[73] found that amyl alcohol is largely eliminated by the lungs in the cat and in the dog. The protocols of his experiments show that 65 per cent of the alcohol given these animals was thus recovered, while he recovered only 22 per cent of this substance in the expired air of the rabbit. Examination of the urine showed the presence of glycuronic acid. Hofmeister's[37] work with tellurium in the dog is of interest in this connection. He made the important discovery that some animals possess the power of methylation as well as of demethylation. Abderhalden and Brahm's [1] experiments with pyridin show that the same is true of young dogs when on a meat diet. His experiments on rabbits with this substance were negative.

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[a] The small figures refer to the bibliography at the end of this bulletin.

The metabolism of caffein and theobromin furnish another illustration of differences in the physiological mechanism of animals. Although the substances found in the urine of man, dog, and rabbit after the administration of caffein and theobromin were the same, the quantities varied considerably. According to Krüger and Schmidt,[47] over 14 per cent of the theobromin introduced into the rabbit is eliminated as 7-methyl xanthin in the urine. The dog eliminates only about 0.67 per cent. On the other hand, the amount of tri-methyl xanthin eliminated was only 3 per cent in the dog and not quite 1 per cent in the rabbit.

It appears, therefore, from studies in comparative metabolism, whether endogenous or exogenous, that well-marked physiologic and chemical differences exist in various species of animals. That pharmacological action may likewise vary in different species of animals is shown by the following investigations. According to Guinard,[31] who made an exhaustive study of morphin, the reaction to this alkaloid varies in different forms of life, both qualitatively and quantitatively. He established its narcotic effect in the dog, rabbit, guinea pig, white mice, and rats, while for the cat, horse, ox, sheep, hog, and goat it is, on the contrary, a stimulant. Moreover, there is no evidence of cerebral effect. The stimulating effect of morphin on the nervous system in some animals was also observed by Noe [65] in experiments with this substance on the hedgehog.

Guinard [29, 30] has also shown that morphin has no narcotic effect in the marmot, although this animal is very sensitive to the drug. Two milligrams per kilo were found to be a surely fatal dose for this animal. His experiments on the comparative toxicity of morphin [30 31] show a considerable range of variation in different species. Thus the fatal dose for the dog was found to be 0.65 per kilo, while 7 mg per kilo is the fatal dose for the horse. About twice the amount is fatal for the ox and 0.2 mg per kilo kills the pig. Experiments with other drugs has shown that a considerable range of variation in resistance exists in animals of different species.

Noe's [65] studies on the comparative toxicity of chloral brought out the interesting fact that the rabbit is more resistant to it than the hedgehog and the latter more resistant than the guinea pig. Perhaps the most striking example of a difference in reaction of the same substance in widely different species is that furnished by apocodein, quinin, and yohimbin. According to Gunn [32] these substances have been found to cause vasodilation in warm-blooded animals, but they constrict the blood vessels of the frog.

Experiments with apomorphin likewise show that the reaction to this substance varies in different species of animals. The resistance of the cat to this drug is, according to Guinard,[31] ten times greater than that of the dog, and the latter is more sensitive than the rabbit to the crystalline form of apomorphin when given intravenously. According to Kobert[45] amygdalin is without effect on dogs, but it is poisonous to rabbits. Lapicque[49] found that the toxicity of curara varies in different species of frogs, the dose required to produce paralysis in *Rana esculenta* being three times greater than in *Bufo vulgaris*. Weir Mitchell[50] pointed out long ago that turtles stand enormous doses of curara. Schmiedeberg's experiments with caffein on *Rana temporaria* and *Rana esculenta* (and more recently those of Jacobi and Golowinski[42] with caffein, theobromin, and theophyllin) are also of interest in this connection. These experimenters observed well-marked differences of reaction to methylxanthins in these closely allied forms.

Experiments with quinin have shown that the action of this substance differs in some animals. It causes a fall of temperature immediately after its administration in the guinea pig, but frequently produces, at first, a rise of temperature, followed by an unimportant fall, in rabbits, dogs, and man.

The numerous investigations which have been carried out on the effect of atoxyl within recent years have contributed much to the comparative pharmacology of this substance. Although the symptoms and organic changes produced by this substance in a variety of animals present no great differences, the resistance of some has been found to vary; according to Köster[46] it is more toxic for dogs than for rabbits. A number of other substances have been found by various experimenters to vary in toxicity for different species of animals. Cantharadin, phenol, atropin, and strychnin may be mentioned as illustrations.

Pharmacological studies on lower forms of life have also revealed marked variations in the effect of some poisons. Observations made by Danilewski[18] with hydrochinone indicate that solutions of 1 to 100 or 200 are toxic to Celentrates, causing paralysis in these organisms. Echinoderms are killed within one or two hours in 1 to 1,000 or 2,000 solution, while in Vermes even weaker solutions cause tetanus and finally paralysis. The experiments of Drzewina[19] with potassium cyanid are also interesting in this connection. Teleosts placed in 100 cc of sea water containing twentieth-normal potassium cyanid showed signs of asphyxia and died in 10 to 20 minutes. Actinia placed in a solution of sea water containing five times as much potassium cyanid were active on the thirteenth day of the experiment. Similar results were obtained with other marine organisms.

From these data it is evident that the toxicity of a substance may vary considerably in different forms of life. It has been shown also by some investigations cited by Salant[78] that the action of drugs may be modified by different conditions in the environment as well as in the subject of the experiment. The recognition of the importance of these factors in determining pharmacological action has contributed much to the elucidation of the mechanism by which drugs and other substances produce physiological effects in the body. Moreover, such knowledge has often enhanced the therapeutic value of pharmaco-dynamic agents and has frequently served to avert effects of an undesirable character in man and domestic animals. The results obtained in one species of animals under a particular condition do not admit, therefore, of universal application. Furthermore, the nature of the action of a drug can only be partly learned from the manifestation of its acute effects. Equally important, therefore, especially in studies on toxicity, are the changes produced in chronic intoxication.

That the acute effects of a substance can hardly be considered a correct estimate of its toxicity is shown by the evidence obtained in experiments on tolerance and cumulative action of drugs; for the toxicity of a substance may diminish when the substance is given steadily for a long time if the body acquires tolerance for it. Arsenic, morphin, and cannabis indica may be cited as illustrations of drugs, the toxicity of which decreases with repeated doses, while digitalis and lead show a tendency to increased toxicity when similarly administered. Moreover the acute and chronic effects are sometimes qualitatively different. According to Igersheimer[41] the symptoms in acute atoxyl intoxication are nausea, vomiting, and diarrhea. These symptoms are absent in chronic intoxication, in which trophic disturbances of the skin and inflammation of the mucous membranes were the effects produced. That the acute action of atoxyl differs from the chronic effects was likewise shown by experiments on animals. The studies of von Anrep[5] on chronic atropin intoxication are of interest in this connection, as he found that after 10 to 15 injections of atropin there is no manifestation of symptoms such as is observed in acute intoxication, while the effects on the circulation are also less marked, the acceleration of the pulse being less than after the same dose in a normal subject not accustomed to its use. When the administration of atropin is continued for a longer time its usual effects on the pulse disappear altogether; there is, on the contrary a decreased frequency of the pulse. If atropin has been administered for from two to three weeks, respiration is likewise affected.

HISTORICAL REVIEW OF THE LITERATURE ON THE TOXICITY OF CAFFEIN.

Caffein was discovered in 1820 by Runge,[77] Pelletier,[69] and Robiquet[76] and was first analyzed by Dumas and Pelletier,[20] but its exact percentage composition was determined by Pfaff and Liebig, [71, 72] while to Herzog [13, 83] belongs the credit of having established that it is basic. Strecker[82] prepared caffein synthetically by heating theobromin silver and methyl iodid in a closed tube for 24 hours. Soon after its discovery in coffee Oudry[67] reported the presence of a substance in tea which he called "thein." Its identity with caffein was established 15 years later by Jobst[43] and also by Mulder. [62, 63] According to Brill,[13] Mulder (1838) was also the first to perform experiments with caffein on animals. After the administration of one-half grain to a pregnant rabbit he observed loss of appetite and kyphosis. The rabbit aborted but recovered from the effects of caffein. It has since been made the subject of numerous investigations which were carried out on a variety of animals. Observations with caffein were also made on the human subject. About four years after Mulder published his results, Lehmann[51] (1842) reported experiments on a number of people who were given caffein. The administration of from 2 to 10 grains of the alkaloid was followed by headache, palpitation of the heart, increased frequency and irregularity of the pulse, tinnitus aurium, photopsia, insomnia, and even delirium. Similar experiments reported by Frerichs[25] (1846) indicate that in doses of 25 grains it may induce severe symptoms about 15 minutes after its administration. He also observed circulatory as well as nervous symptoms and vomiting.

According to Albers[2] (1852), 4.5 grains of caffein citrate injected subcutaneously into the thigh of a rabbit was soon followed by diminished motion and tremors of the operated thigh. Other symptoms reported were spasms of the facial muscles, increased respiratory movements, and mental confusion. Of interest in this connection are the experiments of Cogswell[17] (1852) on frogs. He concluded that in point of destructive action on the tissues, caffein is far superior to morphin and may be compared to strychnin and coniin, its action on the nervous system he believed to be principally confined to the effect on the brain and spinal cord.

Lehmann[52] (1853) observed increased frequency of heart action after the administration of 4 grains, which were given with a normal diet to an adult man. When the dose was doubled the frequency of the pulse was still more increased, heart action became stronger, and tremors and confusion of thought with excitement of the imagination made their appearance. There was also an increased desire to micturate.

Stuhlmann and Falck[83] (1857) were the first to make a study of the toxicity of caffein on animals of different species. The administration of 0.5 gram of caffein subcutaneously or per rectum in rabbits induced tremors, tonic and clonic convulsions, paralysis, and increased frequency of respiration at first followed by violent dyspnœa. On autopsy he noticed congestion of the organs and in two of the three rabbits experimented upon punctiform hemorrhages of the brain with congestion of the meninges were found. In the other rabbit anemia of the brain was observed. Experiments on cats were carried out by subcutaneous, intravenous, and rectal injections. The symptoms observed after the administration of 0.5 to 0.7 gram of caffein were the same as in rabbits except that the cats developed diarrhea when caffein was given and no anatomic lesions were found on autopsy. The effect of caffein on dogs indicated that in subjects of medium weight a dose of 0.5 gram given by mouth might produce restlessness and increased frequency of respiration, while the injection of the same amount intravenously into such animals may cause death. Large, full-grown dogs, however, survived an intravenous injection of 2 grams of caffein, showing symptoms of incoordination, salivation, and frequent defecation. These investigators also made observations on caffein, using pigeons and other birds; 0.5 to 0.1 gram introduced into the stomach caused vomiting, diarrhea, tonic, but more frequently clonic, convulsions, incoordination, tremors, paresis, and paralysis.

In a few, but not in all of the birds, there was at first increased frequency of respiration followed by dyspnœa and circulatory disturbances. These amounts of caffein proved fatal in all of the experiments on birds. Inflammation of the intestinal mucosa and congestion of the meninges were the only changes found on autopsy. Stuhlmann and Falck also studied the effects of caffein on fishes and toads. Mitscherlich[60] (1858) fed 0.4 gram of caffein with bread to a rabbit and noticed lowered temperature, fatigue, convulsions, first increased then decreased frequency of respiration, and on autopsy congestion of all the viscera. He also reported observations on two frogs, one of which was given one-sixteenth of a grain of caffein in a pill with bread. It was administered to the other frog in aqueous solution, but the mode of administration was not published. The symptoms observed were in the main the same as in rabbits. In pigeons 0.125 gram introduced into the stomach caused severe vomiting, muscular incoordination, tonic rigidity of the limbs, and retraction of the head. Respiration was increased in frequency. Death followed within 3 hours and 15 minutes.

From a series of experiments on frogs which Hoppe[38] carried out (1858) by applying one-fourth of a grain of caffein to the muscles of the back, he concluded that caffein causes paralysis of the nerves, spinal cord, and brain, sensation being paralyzed before movement. The injurious action of caffein proceeds, according to Hoppe, from the spinal cord. This was based on experiments on two frogs, *Rana esculenta,* in which the right leg was ampútated, the nerve being left intact, while the nerve of the other leg was ligated. At the end of 30 minutes paralysis was more marked on the right than on the left side. In another frog of the same species he resected the femoral nerve on the right side; about 1½ hours after the administration of caffein convulsions were observed. The left leg was rigid, but the right was relaxed.

Voit[85] (1860) ligated the vessels of the right lower extremity, cut the nerves of the left leg, and introduced a few drops of caffein solution into the stomach. Shortly afterwards tetanus of the right leg occurred on touching the back of the animal; the left leg was motionless. Later the entire body exhibited tetanic convulsions. From this and similar experiments Voit concluded that caffein acts first and principally on the central nervous system, and that caffein is also poisonous to nerve and muscle fibers, as they die when a solution of caffein is applied to them. The action of caffein, according to Voit, is similar in great part to that of strychnin. The effect on the blood vessels is particularly interesting, as Voit observed dilatation of the vessels, due as he thought to muscular paralysis, and also transudation and congestion of the capillaries.

Kurzak[48] (1860) made a study of the comparative toxicity of caffein in frogs and rabbits and came to the conclusion that the lethal dose for frogs is about one-seventh of that for rabbits. Caffein citrate in the form of crystals was administered in both cases by mouth. The doses given to frogs were 1 to 1.5 grains. He observed convulsions and increased respiratory activity at first; after one hour respiration diminished and voluntary muscular activity disappeared. Even on the second day convulsions were sometimes noticed. Death occurred at the end of the first or second day. Experiments on only two rabbits were reported, 0.8 gram of caffein citrate causing the death of one at the end of 13 hours. The symptoms noticed were the same practically as in frogs, but it is interesting to observe that ecchymosis of the mucous membranes of the stomach near the cardia was the only lesion found on autopsy. Several experiments made on different days on the other rabbit indicated that the toxic dose exceeded 0.5 gram, while smaller doses caused but very mild symptoms.

According to Gentilhomme[27] (1867), after caffein the reflexes are at first diminished and then disappear altogether. Death is produced by stiffness and immobility of all the muscles, particularly of the muscles of respiration, thus causing asphyxia. He furthermore held that caffein has no effect on cardiac or smooth muscle fiber, its action being specific on voluntary muscle fiber, contractions of which he observed under the microscope, thus differing completely from strychnin, which is a nerve poison.

These observations seemed to be confirmed by Pratt[74] (1868), who reported that the isolated posterior extremities and muscle fibers of the toad placed in a solution of caffein (1 grain to a wineglassful of water) for three minutes were contracted, while controls placed in distilled water were relaxed. This experiment is, of course, defective, as normal salt solution should have been used in both cases. When the muscular fibers previously immersed in caffein solution were placed under the microscope violent contractions were observed. The same author administered from 2 to 18 grains at a dose to five healthy young men. After the administration of 12 grains he noticed mental anguish, tremors of the hands and arms, and insomnia. Doses under 5 grains had no marked effect except a diminution in the frequency of the pulse and wakefulness.

About the same time Amory[4] (1868) published the results of his studies on the toxicity of caffein in cats, dogs, rabbits, and pigeons. In all cases very large doses were introduced directly into the stomach by means of a temporary gastric fistula. Ten grains given in meat to a dog caused restlessness, but no other symptoms. Doses of 30 grains and above were invariably fatal. Seventy-three grains given to a cat caused death within 20 minutes.

From observations on frogs, guinea pigs, rabbits, and on one dog, Leven[53] (1868) concluded that caffein which he gave in the form of the citrate in doses of 10 mg to frogs, from 150 to 200 mg to guinea pigs, and three to four times the latter amount to rabbits, stimulates the central nervous system and the voluntary, cardiac, and smooth muscles. He found that 0.9 gram caffein was fatal for a rabbit when injected subcutaneously, while 1 gram of the citrate was not toxic for a dog of medium size. Caffein applied directly to muscle fiber causes tetanus and destroys muscular contractility, while a nerve fiber similarly treated loses its irritability.

According to Johansen[44] (1869), caffein acts directly on the muscular fiber. After the subcutaneous injection of 0.02 gram of caffein into frogs, he observed contraction of the muscles at the site of injection, then contraction of the anterior extremities, and finally the posterior extremities become rigid and extended. Johansen observed muscular rigidity after caffein, even after curara was injected, or after ligating the vessels, or cutting the nerves which supply the muscles. He also observed that large doses of caffein diminish muscular irritability. When cardiac muscle was poisoned with caffein, microscopical examination showed that the striations disappeared. Johansen also states that reflexes disappear after caffein poisoning. He never observed tetanus in frogs, but reported tonic and clonic convulsions as a result of caffein poisoning in mammals. Somewhat different effects of caffein in frogs were observed by Buchheim and Eisenmenger[14] (1870). After the injection of 2 per cent of the citrate the frogs soon become inactive. He also observed muscular twitching of the extremities, which gradually increased, with rigidity of the muscles and opisthotonos, while respiration became slow and superficial, finally stopping altogether.

Aubert[6] (1872) studied the toxicity of caffein in man and other animals. After the ingestion of 0.36 gram, he observed dizziness, but doses of 0.12 and 0.24 gram were without any apparent effect. On the other hand, a dose of 0.5 gram of caffein was followed by increased frequency of the pulse, which soon disappeared. After one hour he noticed dizziness and trembling of the hands, which likewise passed away soon. The injection of 0.16 gram of a 2 per cent solution of caffein into the jugular vein of a rabbit weighing 1,090 grams caused tetanus and death in two and one-half minutes, and 0.12 gram injected into a rabbit weighing 980 grams caused death in one minute. Much larger doses could be borne, however, when artificial respiration was resorted to. A dog which was given 3 grams of caffein survived when artificial respiration was performed. Aubert reports, on the other hand, a similar experiment with 0.25 gram of caffein which terminated fatally.

That caffein may give rise to different effects in various species of animals was observed for the first time by Bennett.[9] He studied its action on frogs, mice, rabbits, and cats, and attempted to determine the minimum fatal dose in rabbits and cats. He also reported experiments with thein. In his first communication on the subject he states that the administration of thein to rabbits first increased and then diminished the frequency of respiration, while the pulse was decreased in frequency. Caffein, which he apparently thought was different from thein, caused increased frequency of respiration, while the pulse was markedly retarded after a preliminary acceleration. He also noticed congestion of the ears, muscular incoordination, tetanus, paralysis, diminished reflexes, and contraction of the pupils. Bennett reported the minimum fatal dose of caffein for a rabbit weighing 3.25 pounds as being 5.25–5.5 grains. The symptoms in cats after the administration of toxic doses of thein or of caffein were great excitement, paralysis alternating with convulsions, and profuse salivation. The minimum fatal dose for a cat weighing 5 pounds was, according to Bennett, 6 grains of caffein and 5.5 grains of thein. Only one experiment on a mouse is reported; the administration of 0.1 grain proved fatal. The symptoms were the same as those observed in cats and rabbits after the administration of caffein. The experiments on frogs indicate that the symptoms were about the same as those previously described in the case of warm-blooded animals except that the reflexes are almost completely lost after the subcutaneous injection of doses of one-sixteenth to one-twelfth of a grain. The latter dose was fatal for frogs. It would be of interest to know the comparative toxicity of caffein to frogs and mammals, but unfortunately the weights were not reported.

Schmiedeberg [79] (1874) noticed that the administration of 20 mg of caffein to frogs weighing about 45 grams was followed, in *Rana esculenta,* in about 25 minutes, by increased reflexes, 7 minutes later by tetanus. Several attacks occurred, but tonic spasms were never observed. On the contrary, when the same amount of caffein was given to *Rana temporaria* weighing 45 grams he noticed a marked diminution of the reflexes and tonic rigidity of the muscles after 23 minutes; the reflexes were greatly increased, however, about 24 hours later. The frogs were under observation for three days, and although symptoms were still present at the end of this time in the subjects of both species tetanus was never observed in *Rana temporaria.*

Peretti's [70] (1875) studies on the effects of caffein were confined chiefly to observations on dogs. He also made observations on a few rabbits and reported an experiment on one cat to which he administered, by subcutaneous injection, 0.18 gram of caffein per kilo and noticed increased frequency in lachrymation and crying. The cat was found dead the next day. The subcutaneous injection of a rabbit in which artificial respiration was instituted with 0.36 gram of caffein per kilo proved fatal soon after the injection without any manifestation of symptoms. Small doses of caffein, 0.1 gram, given to a rabbit weighing 3,670 grams, failed to produce any visible effects. Doses under 0.1 gram per kilo likewise failed to induce any symptoms in dogs. When 0.1 gram of caffein per kilo was given by mouth or subcutaneously it was followed by restlessness, salivation, rigidity of hind legs, and vomiting. In both instances the dogs recovered. The symptoms were more severe when the dose was increased to 0.185 gram per kilo, but even in this case the dog recovered. A dose of 0.2 gram per kilo, however, proved fatal.

Henneguy [36] (1875) experimented on three frogs to which he gave 0.01 gram of caffein citrate subcutaneously. He observed mild stimulation of the nervous system and of the muscles, as well as increased cardiac activity. Later, voluntary movement and respiration disappeared and sensations diminished, but convulsions of the extremities appeared. Cardiac activity was then diminished, the heart being finally arrested in systole. Since the motor nerves retained their irritability even after the reflexes disappeared, he concluded that the loss of motion was due to the action of caffein on the nerve centers.

Binz [11] (1878) reported experiments on dogs and also made some observations on man with caffein. The subcutaneous injections of 0.2 gram caffein may prove fatal to dogs, although some survive such a dose. The toxic dose in man varies from 0.5 to 1.5 grams. Disturbance of the circulation, such as palpitation of the heart and fullness of pulse, restlessness, and diarrhea were the symptoms he observed.

Extensive investigations on the action of caffein were carried out by Leblond[50] (1883), who studied its effect on the circulation in man and lower animals, and its toxicity in the lower animals alone. Five to twenty centigrams of caffein and 0.06 to 0.25 gram of salicylate of soda were dissolved and injected into the muscles of the thigh of young guinea pigs weighing a little over 300 grams. In the three experiments reported the death of the animals occurred after 23 minutes, 40 minutes, and 1 hour and 20 minutes. Symptoms appeared in from 10 to 15 minutes after the injection of caffein. Incoordination of movements, convulsions, both tonic and clonic, opisthotonos, tremors, increased frequency of respiration, ataxia, paralysis were the symptoms observed. It is worthy of note that the appearance of paresis preceded the convulsions. Diminished sensation was reported in one pig, but no sensory disturbances nor reflexes had been observed in the other. Two rabbits, one of which received 0.5 and the other about 0.3 gram of caffein per kilo with equal parts of salicylate of soda, were injected subcutaneously into the thigh. Diminished sensation, paresis of the posterior extremities, hyperexcitability, convulsions, opisthotonos, dilation of the veins of the ear were observed. Death followed in 1 hour and 23 minutes in one rabbit and in 3 hours and 7 minutes in the other.

Filehne[22] (1886) experimented with caffein on *Rana esculenta* and *Rana temporaria.* The subcutaneous injection of 7 mg of caffein into *Rana esculenta* caused tetanus, while 50 mg given by mouth caused tonic spasms. He further stated that the difference between *Rana esculenta* and *Rana temporaria* as regards the reaction to caffein was one of degree only.

Amat[3] (1889) reported experiments on three guinea pigs, in which 0.4 to 0.5 gram per kilo injected subcutaneously proved fatal within 38 and 44 minutes. One guinea pig which received 0.1 gram of caffein per kilo survived. The symptoms observed in the two fatal cases were general muscular rigidity and convulsions.

Parisot[68] (1890) made a study of the toxicity of caffein on different species of animals. Unlike most of his predecessors, however, he reported, at least in some cases, the weight of the animals on which he worked. After the subcutaneous and intramuscular injections of from 5 to 20 mg of caffein into *Rana temporaria* weighing from 14 to 16 grams, he noticed increased irritability at first; later, a loss of reflexes, inability to use the muscles, complete muscular rigidity resembling rigor mortis, and also cessation of heart action. The effect of caffein produced in the green frog was analogous to that observed in strychnin poisoning. Parisot found, however, that muscular rigidity developed, although very gradually, also in the green frog, but it set in much later than in frogs of the other species and without superseding the clonic convulsions. According to Parisot, the muscular rigidity after caffein persists after the destruction of the brain and spinal cord, thus showing that it is not of nervous origin. He further emphasized the difference in the behavior of these two species of frogs toward caffein by stating that he never observed tetanic convulsions in the red frog. His experiments also indicate that the green frog is more resistant to caffein than *Rana temporaria,* as the same doses which are fatal for the latter were only toxic for *Rana esculenta.* The number of experiments, however, is too few to justify a positive conclusion on this point. Parisot also made some experiments on turtles. The results he obtained show that caffein is at least as toxic for these animals as for the frogs he experimented upon, 0.33 gram per kilo (carapace not included in weight) having proved fatal within 24 hours. Two experiments on one pigeon were also reported by the same observer; two doses of 0.06 gram per kilo given at an interval of four hours caused mental depression and muscular rigidity, but the pigeon survived.

Experiments with caffein on the human subject made by Parisot showed that man is far more susceptible to this substance than the other animals he investigated. After the ingestion of 0.3 gram of caffein symptoms of intoxication pointing to cerebral disturbance appeared, which became more marked when the size of the doses was increased.

It will be noticed that the nature of the action of caffein, whether it is a nerve or a muscle poison, formed the subject of several investigations. Binz[11] (1890) brought forward additional evidence in support of the view that caffein acts primarily on the ganglion cells, and not on the muscle directly. This he has shown by injecting 0.5 gram into each of two rabbits after cutting the sciatic nerve on one side; in one case he also resected the obdurator and crural nerves on the same side. Clonic spasms developed in both subjects soon after caffein was given, but in each rabbit the side operated upon remained paralyzed. Baldi[8] (1891) studied the action of caffein on *Rana esculenta.* After injecting from 4 to 20 mg tetanus, such as observed in strychnin poisoning, was noticed. Fröhner[26] (1892) made observations on the comparative toxicity of caffein in domesticated animals. After the administration of 5 grams of caffein sodium salicylate by mouth to a dog weighing 10 kilos, he noticed salivation, restlessness, vomiting, and convulsions as in strychnin poisoning. Death occurred three hours after the drug was given. On autopsy he noticed mild inflammation of the mucous membranes of the stomach and intestines and edema of the lungs; the heart was in diastole. A dose of 2 grams of caffein sodium salicylate given to the same animal subcutaneously two days previously provoked only very slight symptoms. The subcutaneous injection of 10 grams of the same preparation into a pig weighing 30 kilos caused death in two and a half hours, with the production of symptoms of disturbance of the nervous system and of gastrointestinal irritation. The same dose per kilo of body weight given to a goat likewise caused death in two and a half hours after its administration. Examination on autopsy revealed inflammation of the gastrointestinal tract. Similar lesions were found in a horse killed by 100 grams of caffein, in which he also noticed hemorrhage of the mucosa in the fundus of the stomach.

Gourewitch [28] (1907) conducted experiments with caffein on rabbits, pigeons, and white rats. It appears from his protocol that single doses of about 0.2 to 0.25 gram caffein per kilo given subcutaneously proved to be fatal. He states, however, that the resistance to caffein was markedly diminished, when its administration was repeated daily, for much smaller amounts sufficed to cause death in these animals. A dose of 120 mg of caffein per kilo proved fatal after the third injection. When the dose was increased to 170 mg per kilo, the animal succumbed to the effects of caffein after the second injection. His experiments on the other animals do not indicate the degree of resistance to caffein, since the weights for some were not given while for the others no attempt was made to determine the minimum toxic or fatal dose.

Maurel [55] (1907) studied the influence of different methods of administration on the toxicity of caffein on frogs and rabbits. He determined the minimum toxic and lethal doses of caffein hydrobromid which he employed in 1 to 2 per cent solutions. He concluded from his experiments that the toxicity of caffein when given by mouth is twice as great for the frog as for the rabbit.

More recently Hale [33] carried out a number of experiments on guinea pigs in which he determined the toxicity of caffein given in the form of the citrate and made into a pill with mucilage of acacia and arrowroot starch. After the pill was dried it was fed to the animal, due precaution being taken that none of it was lost during feeding. From experiments on guinea pigs which received doses of 0.3 to 0.6 gram caffein citrate, the following data have been reported: Three decigrams per kilo given to one pig was not fatal. Of three pigs which received 0.4 per kilo, one died and two survived. Exactly the same results were obtained in three others which received 0.5 per kilo. Two guinea pigs, which received 0.55 and 0.6 per kilo each, died after 15 and 7 hours, respectively, while another animal survived a dose of 0.45 per kilo.

This review of the literature on the toxicity of caffein, although bearing evidence of considerable investigation and extending over three-quarters of a century, is largely qualitative in character. It appears from the experiments that the main object of the investigations was to ascertain the nature of the action of caffein, whether it is a muscle or a nerve poison. The comparative toxicity in different species of animals by the accurate determination of the toxic and fatal doses received but little attention. To fill the gap in our knowledge of the toxic effects of caffein, the present investigation was undertaken. This, it will be seen, proved to be a most laborious task, because in the large number of experiments careful observations showed that individuals of the same species varied considerably in their reaction to the drug. Numerous other factors, as will be shown, were also found to play an important part in the determination of the toxicity of caffein.

ACUTE CAFFEIN INTOXICATION.

The object of these experiments was to determine the resistance to caffein in various species of animals and by various methods of administration. Caffein was therefore given by mouth and injected subcutaneously into the peritoneal cavity, into the muscles, and intravenously. As far as could be judged by appearance, healthy animals were selected for the subjects of the experiments, but as it is impossible to diagnose with any degree of accuracy the condition of the animal while it is alive, post mortem examinations were resorted to in many cases in which the issue of the experiment was fatal. Since the age of the animal may modify toxicity full grown, as well as young, animals were employed for these experiments; diet, race, and season also play an important part in determining the toxicity of a drug and these factors were also taken into account in the present investigation.

EXPERIMENTS ON RABBITS.

Animals of different varieties were used and were given caffein by all of the methods indicated in the preceding paragraph. Some of the rabbits employed in these experiments received oats, others received a diet exclusively of carrots for several days or weeks previous to the administration of caffein. The experiments were conducted at all seasons of the year.

SUBCUTANEOUS INJECTION.

From a study of the literature on the toxicity of caffein it seemed that about 150 mg per kilo is probably the lethal dose for the rabbit when the drug is injected subcutaneously. Preliminary observations were therefore carried out with such a dose, but it was found, on the contrary, that this amount per kilo was hardly sufficient to induce symptoms in the great majority of cases.

SERIES A.

[Doses of 147 to 167 mg of caffein per kilo were employed in these experiments.]

Rabbit 332. Belgian hare, female. Weight, 1,070 grams. Diet, oats.
March 25: 8.5 cc 2 per cent caffein (158 mg per kilo) injected subcutaneously at 2.15 p. m.; 4 p. m., reflexes increased; 5.45 p. m., increases of reflexes still more marked.
March 26: Rabbit looked normal; no symptoms observed.

Rabbit 331. Belgian hare, female. Weight, 1,170 grams. Diet, oats.
March 25: 2.15 p. m., 9 cc 2 per cent caffein (153 mg per kilo) injected subcutaneously; 4 p. m., reflexes increased; 5.45 p. m., condition the same.
March 26: Rabbit looks normal; no symptoms observed.

Rabbit 328. Belgian hare, female. Weight, 1,200 grams. Diet, oats.
March 25: 9 cc 2 per cent caffein injected subcutaneously (150 mg per kilo); 4 p. m., reflexes increased; 5.45 p. m., reflexes increased but not markedly.
March 26: No symptoms; rabbit looks normal.

Rabbit 322. White female. Weight, 1,065 grams. Diet, oats.
March 17: 8 cc 2 per cent caffein (150 mg per kilo) injected subcutaneously at 11.55 a. m.; 12.55 p. m., reflexes increased, but no tetanus nor any other symptoms.
March 18: Rabbit running around in cage; condition apparently normal.
March 25: Condition of rabbit good.

Rabbit 217. White. Weight, 1,355 grams. Diet, oats.
October 29: 10 cc 2 per cent caffein (147 mg per kilo) injected subcutaneously at 1.51 p. m. 5.15 p. m., rabbit alive; survived.

Rabbit 219. Maltese. Weight, 1,820 grams. Diet, oats.
October 29: 14 cc 2 per cent caffein injected subcutaneously at 1.40 p. m. (153 mg per kilo); 5.15, rabbit alive; survived.

Rabbit 194. White female. Weight, 1,490 grams. Diet, oats.
October 14: 13 cc 2 per cent caffein (174 mg per kilo) injected subcutaneously; increased reflexes and tremors were observed.
October 15: Condition of rabbit good; no symptoms.

Rabbit 191. Brown male. Weight, 1,915 grams. Diet, oats.
October 14: 16 cc 2 per cent caffein (167 mg per kilo) injected subcutaneously; reflexes increased and tremors present.
October 15: Condition of rabbit good.

A study of this series shows that about 150 mg of caffein per kilo caused increased reflexes within one to two hours after injection. When the dose was increased, as in rabbits 194 and 191, the symptoms were more pronounced; 150 mg per kilo may be regarded as the minimum dose which produces symptoms of nervous irritability when caffein is injected subcutaneously. Experiments with larger doses were therefore carried out in order to determine the minimum fatal dose.

SERIES B.

Approximately 0.2 gram of caffein per kilo was employed in these experiments. Diet and race as possible factors which may influence the toxicity of caffein were made the subject of study in these experiments which were divided into two groups as shown in the table, page 25.

Rabbit 95. Gray and white male. Weight, 1,478 grams. Diet, oats.
February 27: 11.30 a. m., 15 cc 2 per cent caffein (210 mg per kilo) injected subcutaneously; 2.20 p. m., no symptoms, tremors observed when handled, but not marked, reflexes slightly increased, no muscular rigidity nor any other symptoms; 2.45 p. m., rabbit suddenly became very restless, jumped off the table, and had convulsions; 3.45 p. m., rabbit died, rigor mortis set in almost immediately after death.

Rabbit 96. Gray and white male. Weight, 1,585 grams. Diet, oats.
February 27: 16 cc 2 per cent caffein (200 mg per kilo) injected subcutaneously at 3.40 p. m.; increased reflexes observed about one hour after caffein was injected, but no other symptoms.
February 28: Rabbit found dead.

Rabbit 112. Black female. Weight, 875 grams. Diet, oats.
March 18: 9 cc 2 per cent caffein (205 mg per kilo) injected subcutaneously at 3 p. m.; 3.30 p. m., rabbit became restless, reflexes were increased, tremors were

observed, but no other symptoms; 4.15 p. m., rabbit had tremors, was handled but this failed to induce tetanus, 10 minutes later tetanus of short duration with recovery occurred.

March 19: 9 a. m., found dead.

Rabbit 119. Yellow white female. Weight, 1,060 grams. Diet, oats.

April 17: 10 cc 2 per cent caffein (188 mg per kilo) injected subcutaneously at 2.10 p. m.

April 18: Rabbit found dead.

Rabbit 195. White female. Weight, 1,300 grams. Diet, carrots, since October 7.

October 14: 13 cc 2 per cent caffein (0.2 gram per kilo) injected subcutaneously at 11.15 a. m.; 2.25 p. m., rabbit had convulsions and died. *Note:* Ulceration of rectum was noticed.

Rabbit 208. Gray. Weight, 1,068 grams. Diet, carrots, October 7-15, inclusive.

October 15: 10 cc 2 per cent caffein injected subcutaneously at 11 a. m.; 1 p. m., increased reflexes and tremors observed; 3.45 p. m., tremors were marked when rabbit was handled.

October 16: Rabbit found dead. *Note:* Looked poorly nourished.

Rabbit 247. Belgian hare, female. Weight, 1,295 grams. Diet, oats last 10 days before experiment.

November 10: 11 a. m., urine obtained from the bladder was acid to litmus and did not contain sugar or albumen, 13 cc 2 per cent caffein was injected subcutaneously; 1.30 p. m., 15 cc urine obtained was markedly alkaline to litmus and reduced Fehling's solution; 2.30 p. m., reduction of urine considerable, marked tremors observed but no tetanus.

November 11: 10.30 a. m., 95 cc urine collected gave moderate reduction of Fehling's solution, no symptoms, condition of rabbit seemed to be good.

Rabbit 248. Belgian hare, female. Weight, 1,305 grams. Diet, oats the last 10 days before the experiment.

November 10: 11 a. m., urine markedly acid to litmus, no albumen, no sugar; 13 cc 2 per cent caffein injected subcutaneously; 1.30 p. m., urine was slightly alkaline to litmus, no reduction of Fehling's solution; 2 p. m., reflexes increased; 2.30 p. m., 2 cc urine obtained from bladder, sugar abundant; 4.45 p. m., reflexes increased as before, but no tetanus.

November 11: 10.30 a. m., urine collected showed slight reduction of Fehling's solution; otherwise condition of rabbit was good; rabbit did not show any effects of caffein.

Rabbit 337. Belgian hare. Weight, 1,040 grams. Diet, carrots, March 31 to April 6, inclusive.

April 6: 3 p. m., 11 cc 2 per cent caffein injected subcutaneously in the back (0.211 per kilo); 4.30 p. m., reflexes much exaggerated.

April 7: 8.15 a. m.; condition good, no symptoms.

Rabbit 336. Belgian hare. Weight, 1,040 grams. Diet, carrots, March 31 to April 6, inclusive.

April 6: 3 p. m., 11 cc 2 per cent caffein injected subcutaneously into tissues of the back.

April 7: 8.15 a. m., no symptoms, condition good.

Although symptoms appeared in rabbits of Group I (see table, page 25) about the same time after the administration of caffein as in the rabbits of the preceding series all of them terminated fatally 2¼ hours to 24 hours after its administration. Two of these rabbits (Nos. 195 and 208) were fed carrots for several days before the injection of caffein, the others were fed oats. Since symptoms and death appeared in these two rabbits about the same time as in the rest of this group it may be concluded that caffein is not less toxic when carrots are fed than when oats form the exclusive diet. But since rabbit No. 208 was poorly nourished and ulceration of the rectum was observed in No. 195 it is quite possible that caffein might be less toxic in normal rabbits on this diet. This was tested in rabbits Nos. 336 and 337, both of which seemed to be free from abnormality and were well nourished. Since these rabbits survived and manifested mild symptoms only of intoxication it would seem that a carrot diet decreases the toxicity of caffein.

It was suggested, however, that another factor might be the cause of the greater resistance to caffein in these two rabbits, namely, race. This was tested in rabbits 247 and 248, both Belgian hares. Since the toxicity of caffein in these two rabbits was the same as in Nos. 336 and 337, diet as a factor in acute caffein intoxication may be disregarded. The greater resistance to caffein of these four rabbits is in all probability due, therefore, to a difference of race. This suggestion gained additional support from the experiments of the next series.

SERIES C.

The object of these experiments was to determine the minimum fatal dose for the gray rabbit and to obtain additional evidence as to the toxicity of caffein in the several varieties of rabbits. Eight experiments were performed, in which from 236 to 252 mg per kilo were given. The white rabbits, three in number, received 250, 242, and 238 mg per kilo. All the others (which were Belgian hares) received from 236 to 252 mg per kilo. Two of the white rabbits were fed carrots for one week preceding the injection of caffein. The other was fed oats. Three of the Belgian hares were on a diet of oats, two were fed carrots the week before the experiment with caffein.

Rabbit 122. White, female. Weight, 2,060 grams. Diet, oats.

April 14: 25 cc of 2 per cent caffein (250 mg per kilo) in aqueous solution injected subcutaneously in the back at 1.35 p. m.; 4.30 p. m., tremors, reflexes increased, condition otherwise good.

April 16: 9 a. m., found dead in cage. *Autopsy:* Liver deeply congested; kidneys congested in cortex and medulla; stomach showed small hemorrhagic areas, perforating ulcers in pyloric portion; small intestine petechiated on mucosa; lungs and spleen normal.

Rabbit 234. White, female. Weight, 1,650 grams. Diet, November 2–9, carrots.

November 9: 10.45 a. m., 20 cc 2 per cent caffein (242 mg per kilo) administered subcutaneously.

November 10: 9 p. m., found dead.

Rabbit 335. Gray hare, female. Weight, 1,170 grams. Diet, March 31 to April 7, carrots.

April 7: 9.30 a. m., 14 cc 2 per cent caffein solution (240 mg per kilo) injected subcutaneously in the back; 10.30, reflexes much increased, rabbit is extremely sensitive.

April 8: 9 a. m., found dead. *Autopsy:* Liver was congested and contained several coccidiosis nodules; stomach distended with rather dry food mass; mucosa exhibited mild catarrhal inflammation; mucosa of intestines also slightly inflamed.

Rabbit 249. Belgian hare, female. Weight, 1,185 grams. Diet, oats.

November 11: Urine, 5 cc, from bladder acid to litmus, no sugar, no albumin; 11.50 a. m., 14 cc 2 per cent caffein (236 mg per kilo) administered subcutaneously; 3.45 p. m., reflexes increased, hyperæsthesia marked, but no tetanus, even when handled; 30 cc urine collected at 4 p. m., reduction of Fehling's solution considerable.

November 12: 10 a. m., 8 cc urine collected, reduction heavy, only a few cubic centimeters obtained from bladder, did not contain any sugar, general condition of rabbit good, no symptom of caffein intoxication.

Rabbit 321. Yellow, female. Weight, 1,135 grams. Diet, oats.

March 16, 1910: 11.50 a. m., 14 cc 2 per cent caffein (246 mg per kilo) injected subcutaneously in the back; 2 p. m., reflexes increased, is very sensitive, started to run when put on floor, no handling except what was required for removal and return to cage, feces soft.

March 17: 9.30 a. m., condition good, rabbit put on floor, gait normal, but does not care to walk.

March 18: 9 a. m., walks around when put on floor, appetite good, condition seems to be normal.

March 25: 11 a. m., rabbit still alive, condition good.

Rabbit 250. Belgian hare, female. Weight, 1,435 grams. Diet, oats at least two days before the experiment.

November 11: 11 a. m., urine obtained from bladder acid to litmus, no albumin, no sugar; 11.10 a. m., 18 cc, 2 per cent caffein (252 mg per kilo); 3.45 p. m., reflexes and hyperæsthesia, no tetanus; 4 p. m., 60 cc urine, marked reduction of Fehling's solution.

November 12: 10 a. m., condition of rabbit good, no symptoms of caffein intoxication, 80 cc urine collected, sugar considerable, only a few cubic centimeters of urine obtained from bladder, no reduction of Fehling's solution.

Rabbit 334. Belgian hare, female. Weight, 1,270 grams. Diet, carrots, March 31 to April 7.

April 7: 9.30 a. m., 15 cc 2 per cent caffein (240 mg per kilo) injected subcutaneously in the back; 10.30 a. m., reflexes much increased, rabbit extremely sensitive.

April 8: 9 a. m., condition good, no symptoms.

Rabbit 233. White, male. Weight, 1,675 grams. Diet, carrots, November 2 to 9.

November 9: 10.50 a. m., 20 cc 2 per cent caffein (238 mg per kilo) injected subcutaneously, no symptoms observed until 5 p. m., when increased reflexes and hyperæsthesia were noticed, but no tetanus.

November 10: 9 a. m., paralysis of posterior extremities; died at 1 p. m.

Analysis of the results obtained in the experiments of this series and inspection of Table I, page 25, show that all four of the rabbits which survived doses of 236 to 252 mg of caffein per kilo were Belgian hares. Of the four which died one only was a Belgian hare. The other three were white rabbits. Two of these were fed oats; the other two received carrots during seven days preceding the administration of caffein. This diet does not seem to be a factor, therefore, in the toxicity of caffein. Moreover, it may be observed that rabbit No. 122, which was fed oats, died after receiving 250 mg per kilo, while rabbit No. 250 received the same diet and survived the same dose of caffein per kilo.

Experiments 234 and 334 offer another illustration that the toxicity of caffein is not dependent upon diet, since both rabbits were fed carrots, but the same dose of caffein caused only symptoms in one while it proved fatal to the other. It is evident, therefore, that the difference in resistance to caffein shown in these experiments is in all probability due to race, the Belgian hare being more resistant to caffein than rabbits of other varieties. Rabbit No. 335 seems to be an exception, but the post-mortem examination showed the presence of coccidiosis of the liver. As will be shown later, wherever this condition prevailed even smaller doses of caffein proved fatal.

SERIES D.

To obtain additional evidence regarding the resistance of the various races of rabbits to caffein and to ascertain the smallest dose which is surely fatal to the gray rabbit or Belgian hare was the object of this series of experiments. The diet in all cases consisted of oats, which was given ad libitum excepting to rabbit No. 235, which received carrots for one week previous to the injection of caffein. The doses administered ranged from 267 to 300 mg per kilo and were administered to different varieties of adult rabbits.

Rabbit 253. Brown and black, male. Weight, 1,600 grams. Diet, oats, November 9 to 12.

November 12: 11.30 a. m., urine from bladder acid, no albumen, no sugar; 11.35 a. m., 22 cc 2 per cent caffein (275 mg per kilo) injected subcutaneously; 11.45 a. m., rabbit jumped off the table, had convulsions, retraction of head and opisthotonos, general tremors, anterior extremities stretched out, posterior extremities almost normal, frequent twitchings; died at 12.15 p. m.

Rabbit 252. Black, female. Weight, 1,335 grams. Diet, oats, November 9 to 12.

November 12: 11.30 a. m., 18 cc 2 per cent caffein (270 mg per kilo) injected subcutaneously. Urine obtained from bladder before injection, acid, no albumen, no sugar, color normal, tremors and great excitement noticed about 12 noon; 4.30 p. m., when handled, showed unusual restlessness and excitement followed by convulsions with opisthotonos; occasional twitching, condition bad. Died 4.35 p. m.

Rabbit 327. White, female. Weight, 820 grams. Diet, oats, March 8 to 16.

March 16: 11.45 a. m., 12 cc 2 per cent caffein (292 mg per kilo) injected subcutaneously in the back; 2 p. m., found dead, but was still warm. *Autopsy:* Hemorrhagic area at point of injection into spinal muscles; subcutaneous abdominal region exhibited a large area of cheesy purulent material; liver and spleen were engorged; bladder filled; intestines normal.

Rabbit 340. White and brown male. Weight, 1,465 grams. Diet, oats.

March 30: 3.20 p. m., 20 cc of 2 per cent caffein (273 mg per kilo) injected subcutaneously in back.

March 31: 9 a. m., found dead.

Rabbit 341. White and brown. Weight, 1,450 grams. Diet, oats.

March 30: 3.20 p. m., 20 cc 2 per cent caffein (270 mg per kilo) injected subcutaneously in back; 4.40 p. m., found in dying condition, had convulsions; 4.45 p. m., dead.

Rabbit 326. White, male. Weight, 1,645 grams. Diet, oats, March 8 to 16.

March 16, 1910: 12 noon, 20 cc 2 per cent caffein (243 mg per kilo) injected subcutaneously in the back; 2 p. m., tremors marked, hypersensitive, started to run when put on floor; rabbit was not handled any more than was required for his removal from and return to cage.

March 17: 9.30 a. m., tremors still present and marked, otherwise general condition good; no other symptoms.

March 18: 9.30 a. m., no appetite, tremors still present, general condition poor; died about 2 p. m.

Rabbit 235. Belgian hare, male. Weight, 1,870 grams. Diet, carrots, November 2 to 9.

November 10: 11.05 a. m., 25 cc 2 per cent caffein (267 mg per kilo) injected subcutaneously; reflexes increased and tremors, but no tetanus observed; found dead next morning.

Rabbit 316. Belgian hare, female. Weight, 860 grams. Diet, oats, March 8 to 16.

March 16, 1910: 11.40 a. m., 12 cc 2 per cent caffein (267 mg per kilo) injected subcutaneously in the back; 2.15 p. m., reflexes somewhat increased, but not markedly so; walked when put on floor; gait clumsy and slow; tremors of head observed; 2.35 p. m., rabbit lying in his cage, posterior extremities extended and rigid, anterior extremities flexed, head retracted; is still breathing; occasional spasms observed. Rabbit died at 3 p. m. *Autopsy:* No lesion at point of injection in dorsal spinal muscles; liver and spleen engorged; intestines injected; other organs apparently normal.

Rabbit 395. Belgian hare, male. Weight, 1,410 grams.

August 18: 1 p. m., 20 cc 2 per cent caffein (283 mg per kilo) injected subcutaneously in the back; 4 p. m., reflexes markedly increased; 5 p. m., reflexes about the same, but no tetanus.

August 19, 9.15 a. m.: Reflexes increased markedly.

August 21, weight, 1,215 grams. Given 275 mg per kilo of caffein; no symptoms observed.

August 23, found dead. *Autopsy:* Liver greatly engorged; stomach fairly well distended and mucous membrane in a slightly inflammatory condition; contents of small intestine liquid in nature, but walls of same appeared normal; other organs normal in appearance.

Rabbit 396. Belgian hare, female. Weight, 1,475 grams. Diet, oats.

August 18: 1 p. m., 20 cc 2 per cent caffein (272 mg per kilo) injected subcutaneously in the back; 4 p. m., reflexes increased markedly; 5 p. m., reflexes increased markedly but no tetanus.

August 15: 10.30 a. m., reflexes still increased very markedly; rabbit jumps when touched.

August 21: Weight, 1,245 grams. Injected subcutaneously 275 mg of caffein per kilo; reflexes increased, posterior extremities stiff over hour later.

August 22: 9 a. m., found dead. *Autopsy:* Thoracic organs normal in appearance; stomach distended and mucous membrane affected with a catarrhal inflammation; contents of stomach were covered with a shiny mucus; contents of small intestine liquid in nature and bile stained; liver showed a coccidial infestation; kidneys and spleen normal in appearance.

Rabbit 397. Belgian hare, male. Weight, 1,375 grams. Diet, oats.

August 19: 10.30 a. m., 20 cc 2 per cent caffein (290 mg per kilo) injected subcutaneously in the back.

August 22: 9 a. m., found dead. *Autopsy:* Stomach distended with ingesta; mucous membrane exhibited a catarrhal inflammation with excessive secretions; major portion of intestines showed a condition similar to that of stomach, contents consisting mainly of a shiny mucus; liver enlarged; other organs apparently normal.

Rabbit 398, Belgian hare, female. Weight, 1,570 grams. Diet, oats.

August 19: 10.30 a. m., 23 cc 2 per cent caffein (293 mg per kilo) injected subcutaneously in the back; 4 p. m., found dead. *Autopsy:* Thoracic organs seemingly normal; mucous membrane of stomach exhibited a catarrhal inflammation generally; large intestines somewhat impacted but walls appeared normal; other organs normal.

Rabbit 399, Belgian hare, male. Weight, 1,725 grams. Diet, oats.

August 19: 10.30 a. m., 26 cc 2 per cent caffein (300 mg per kilo) injected subcutaneously in the back; found dead at 4.30 p. m. *Autopsy:* Lungs slightly congested; liver engorged and friable; gall cyst well filled; stomach exhibited catarrhal gastritis; injection of mesenteries and intestines; kidney showed marked cortical congestion.

The results of the experiments of this series likewise indicate that the Belgian hare is more resistant to caffein than the rabbits of other varieties. Thus, of the four gray rabbits (Nos. 235, 316, 395, and 396), which received 267 to 283 mg of caffein per kilo, two died and two lived,[a] one of which, 396, showed the presence of coccidiosis of the liver. On the other hand it will be observed that the black and white rabbits which received from 270 to 275 mg of caffein per kilo all died from the effects of the drug; one within 1 hour and 25 minutes and another within 50 hours after the administration of the caffein, while No. 340 died in the night. Furthermore it will be noted that of the last three rabbits of this series, which were Belgian hares and received 290, 293, and 300 mg of caffein, two died six hours after the injection, while the other, No. 397, lived three days. The minimum fatal dose of caffein for Belgian hares is, therefore, about 290 to 300 mg per kilo when injected subcutaneously, which is about 50 per cent greater than for rabbits of other varieties.

SERIES E.

It was shown in series A that 0.15 caffein per kilo caused symptoms of intoxication. Before concluding, however, that this is the smallest dose which causes symptoms of poisoning, a number of experiments were performed with smaller doses. It was found that in the great majority of cases 0.1 caffein per kilo may cause diuresis, but no nervous or muscular symptoms. In some rabbits, however, even such a dose proved fatal. Post-mortem examinations in these cases showed the presence of coccidiosis of the liver, and it will be recalled that similar observations were made before. It is quite possible, therefore, that coccidiosis of the liver is an important factor in decreasing the resistance to caffein. Experiment 551 (p. 25) shows that other conditions may likewise increase the toxicity of caffein.

Rabbit 325. White, female. Weight, 1,065 grams. Diet, oats.

March 17: 11 a. m., 6 cc 2 per cent (112 mg per kilo) caffein injected subcutaneously in the back. About 5 cc of urine squeezed out from bladder before injecting caffein.

March 17: 1 p. m., hind legs crossed and stretched out, front legs also extended; rabbit lying stretched out on her belly.

March 17: 5.40 p. m., rabbit still alive, condition somewhat improved.

March 18: 9 a. m., found dead, stiff and cold. *Autopsy:* Hemorrhagic area at point of inoculation; subcutaneous region of both thighs presented a hemorrhagic infiltration of the tissues; liver contained lesions of coccidiosis; other organs apparently normal.

Rabbit 330. Belgian hare, female. Weight, 935 grams; poorly nourished.

March 18: 3.35 p. m., 5 cc 2 per cent caffein (107 mg per kilo) injected into subcutaneous tissues in the back; 5.30 p. m., no symptoms.

March 19: 9 a. m., no symptoms.

March 25: Weight, 825 grams.

Rabbit 329. Belgian hare, male. Weight, 775 grams; poorly nourished. Received March 18.

March 18: 3.30 p. m., 4 cc 2 per cent caffein (103 mg per kilo) injected into subcutaneous tissues in the back; 5.30 p. m., no symptoms.

March 19: 9 a. m., no symptoms.

March 25: Rabbit alive in good condition; weight, 825 grams.

Rabbit 320. Black, male. Weight, 1,040 grams. Diet, oats.

March 17: 11 a. m., 6 cc 2 per cent caffein (115 mg per kilo) injected subcutaneously in the back; only a few drops of urine obtained from bladder before injecting caffein; 1 p. m., rabbit very restless; ran away when placed on floor; cried when touched with a piece of paper; no tremors observed, but rabbit became exhausted and was unable to walk; legs extended out; after running for about a minute dyspnœa was very marked, but rabbit soon raised himself on his legs; 5.40 p. m., rabbit up on his legs.

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[a] Survived first dose.

March 18: 9 a. m., found dead, but still warm. *Autopsy:* Lungs studded with small grayish white nodules, adhesions to costal pleura; probably lesions of coccidiosis; liver studded with coccidiosis nodules. Hemorrhages at point of inoculation.

Rabbit, 551. Gray, female. Weight, January 26, 1,650 grams. Diet, oats; fed 20 cc of 25 per cent alcohol daily from January 26–31.

January 31: Weight, 1,450 grams; 10.20 a. m., temperature 101.6°; 10.45, a. m., temperature 101.6°; received 7 cc 2 per cent caffein subcutaneously into back; 11.15 a. m., convulsions of short duration; raised himself on posterior legs, anterior legs wide apart; 4.10 p. m., looked normal, not hypersensitive; 4.30 p. m., condition seemed to be good.

February 1: 9 a. m., found dead, was alive at 5.30 p. m. of previous day. *Autopsy:* Lesions found involved thoracic cavity mainly; lungs were hepatized and a fibro plastic exudate caused them to adhere to costal pleura; liver engorged and appeared fatty; no marked lesions affecting digestive tract, a slight catarrh of stomach being the only noticeable feature; kidneys and spleen normal.

TABLE 1.—*Subcutaneous injections of caffein—rabbits.*

SERIES A.

No.	Weight.	Caffein per kilo.	Appearance of symptoms in—	Duration of life.	Diet.	Remarks.
	Grams.	*Mg.*				
332	1,070	158	1 hour 45 minutes	Survived	Oats	Gray.
331	1,170	153	do	do	do	Do.
328	1,200	150	do	do	do	Do.
322	1,065	150	1 hour	do	do	White.
217	1,355	147		do	do	Do.
219	1,820	153		do	do	Maltese.
194	1,490	174		do	do	White.
191	1,915	167		do	do	Light brown.

SERIES B, GROUP I.

No.	Weight.	Caffein per kilo.	Appearance of symptoms in—	Duration of life.	Diet.	Remarks.
95	1,478	210	2 hours 50 minutes	3 hours 10 minutes	Oats	White.
96	1,585	200	1 hour	About 18 hours	do	Gray white.
112	875	205	30 minutes	do	do	Black.
119	1,060	188		do	do	Yellow white.
195	1,300	200		3 hours 10 minutes	Carrots	White.
208	1,068	188	2 hours	About 24 hours	do	Gray.

SERIES B, GROUP II.

No.	Weight.	Caffein per kilo.	Appearance of symptoms in—	Duration of life.	Diet.	Remarks.
247	1,295	200	2.5 hours	Survived	Oats	Gray.
248	1,305	200	3 hours	do	do	Do.
337	1,040	211	1.5 hours	do	Carrots	Do.
336	1,045	211	do	do	do	Do.

SERIES C.

No.	Weight.	Caffein per kilo.	Appearance of symptoms in—	Duration of life.	Diet.	Remarks.
122	2,060	250	2 hours 55 minutes	1.5 days	Oats	White.
234	1,650	242		About 24 hours	Carrots	Do.
335	1,170	240	1 hour	do	do	Gray coccidiosis.
249	1,185	236	4 hours	Survived	Oats	Gray.
321	1,135	246	2 hours 10 minutes	do	do	Yellow.
250	1,435	252	4 hours 35 minutes	do	do	Gray.
334	1,270	240	1 hour	do	Carrots	Do.
233	1,675	238	6 hours 10 minutes	26 hours	do	White.

TABLE 1.—*Subcutaneous injections of caffein—rabbits*—Continued.

SERIES D.

No.	Weight.	Caffein per kilo.	Appearance of symptoms in—	Duration of life.	Diet.	Remarks.
	Grams.	*Mg.*				
253	1,600	275	10 minutes	35 minutes	Oats	Brown and black.
252	1,335	270	30 minutes	4 hours 55 minutes	...do	Black.
327	820	292		2 hours 15 minutes	...do	White.
340	1,465	273		About 18 hours	...do	White and brown.
341	1,450	270		1 hour 25 minutes	...do	Do.
326	1,645	243	2 hours	50 hours	...do	White.
235	1,875	267		20 hours	Carrots	Gray.
316	860	267	2 hours 45 minutes	3 hours 20 minutes	Oats	Do.
395	1,410	283	3 hours	Survived		Do.
395	1,215	275	.....do	About 2 days	Oats	Do.
396	1,475	272	.....do	Survived	...do	Do.
396	1,245	275	1 hour	About 18 hours	...do	Do.
397	1,375	290		3 days	...do	Do.
398	1,570	293		5.5 hours	...do	Do.
399	1,725	300		6 hours	...do	Do.

SERIES E.

325	1,065	112	2 hours	Less than 22 hours	Oats	White female.
330	935	107	None	Survived		Gray.
329	775	103	.....do	.....do		Gray male.
320	1,040	115	2 hours	46 hours	Oats	Black male.
551	1,450	100	30 minutes	Less than 24 hours	...do	Gray female.

ADMINISTRATION BY MOUTH.

These experiments were carried out on two varieties of rabbits, the white and the gray. The diet consisted chiefly of oats, but in a few cases carrots formed the exclusive diet. Food and water were given ad libitum. A 2 per cent solution of caffein was administered through a stomach tube. Since the resistance to most drugs is commonly supposed to be greater when given by mouth than when administered by any other path, doses of 175 to 200 mg per kilo were fed in a series of preliminary experiments, all of which were performed on gray rabbits weighing from 865 to 1,135 grams, and which were fed carrots for several days previous to the experiment. Three of the rabbits survived, two without showing any symptoms; in the other case paralysis of the posterior extremities was observed five hours after he received caffein and he was found dead the next morning. Unfortunately no autopsy was performed. The low resistance to caffein of this animal was probably due to some abnormal condition which developed about the time of the experiment, since this rabbit received 325 mg of caffein per kilo two weeks previously and increased reflexes only were observed as a result of this treatment. Hence 200 mg of caffein per kilo can not be considered the toxic dose when fed by mouth. In the following experiments larger doses were therefore given.

SERIES A.

Rabbit 248. Belgian hare. Weight, 1,170 grams. Diet, oats.

November 17: 1.20 p. m., 19.5 cc 2 per cent caffein (330 mg per kilo) administered by the mouth; 4.30 p. m., somewhat hypersensitive.

November 19: No symptoms; at 9 a. m., urine collected, no reduction of Fehling's solution; rabbit survived.

Rabbit 241. White male. Weight, 1,380 grams. Diet, oats.

November 17: 1.15 p. m., 20 cc 2 per cent caffein (290 mg per kilo) administered by the mouth; 4.30 p. m., some hypersensitiveness, but no other symptoms.

November 18: 9 a. m., urine collected, no reduction of Fehling's solution; no symptoms; rabbit survived.

Rabbit 249. Belgian hare. Weight, 890 grams. Diet, oats.

November 17: 1.30 p. m., 14.5 cc 2 per cent caffein (325 mg per kilo) administered; 4.30 p. m., hypersensitiveness; no other symptoms.

November 18: 10 a. m., no symptoms; urine collected, no reduction; rabbit survived.

SERIES B.

The object of these experiments was to determine the minimum fatal dose of caffein in the two varieties of rabbits, the white and the gray. All of the animals selected were approximately of the same weight.

Rabbit 239. Belgian hare, male. Weight, 935 grams. Diet, oats.

November 19: 4 p. m., 17 cc 2 per cent caffein (363 mg per kilo) administered by mouth, followed by 10 cc of 0.9 per cent salt solution.

November 20: Urine examined, no sugar found, no symptom noticed at any time after injection.

Rabbit 254. Belgian hare, female. Weight, 975 grams. Diet, oats.

November 19: 4.05 p. m., 18 cc 2 per cent caffein (369 mg per kilo) administered by mouth, followed by 10 cc of 0.9 per cent salt solution.

November 20: 9 a. m., rabbit found dead.

Rabbit 267. White. Weight, 1,050 grams. Diet, oats.

November 23: 12.10 p. m., 18 cc 2 per cent caffein (342 mg per kilo) given by mouth, followed by 18 cc salt solution; 1 p. m., increased reflexes, tremors marked but no tetanus; 1.05 p. m., rabbit stretched on abdomen, posterior extremities in extended position and paralyzed, soon after clonic spasms set in, which recurred about every minute; 1.14 p. m., tetanus and death. *Autopsy:* Liver showed fatty degeneration; slight inflammation of stomach and intestines; other organs normal.

Rabbit 268. White. Weight 1,100 grams. Diet, oats.

November 23: 20 cc 2 per cent caffein (363 mg per kilo) administered by mouth, followed by 20 cc salt solution; 1.15 p. m., somewhat hypersensitive; 4.30 p. m., tremors fairly marked, no urine passed, about 2 cc of bloody looking urine obtained from bladder, which contained albumen and a considerable amount of glycogen; rabbit died.

Rabbit 419, Belgian hare, male. Weight, 1,600 grams. Diet, oats.

September 26: 10 a. m., 28 cc 2 per cent caffein (350 mg per kilo) given by mouth; reflexes increased at 4 p. m.; 6 p. m., reflexes still increased, no other symptoms.

September 27: 9 a. m., found dead. *Autopsy:* Lungs, liver, and kidneys congested; other organs normal.

Rabbit 420. Belgian hare, male. Weight, 1,250 grams. Diet, oats.

September 26: 10 a. m., 22 cc 2 per cent caffein (352 mg per kilo) given by mouth; 11.35 a. m., convulsions; 12 noon, found dead. *Autopsy:* Liver showed very extensive coccidiosis; no other lesions.

Rabbit 421. Belgian hare, male. Weight, 1,485 grams. Diet, oats.

September 26: 10 a. m., 26 cc 2 per cent caffein (351 mg per kilo) administered by mouth; 4 p. m., reflexes increased; 6 p. m., reflexes as before, no tetanus observed.

September 27: 9 a. m., rabbit found dead. *Autopsy:* Congestion of lungs and kidneys; liver congested and slightly fatty.

Rabbit 424. White, male. Weight, 1,295 grams. Diet, oats.

September 26: 2 p. m., 19 cc 2 per cent caffein (293 mg per kilo) administered by mouth; 4 p. m., reflexes increased, no other symptoms; 6 p. m., no change since 4 p. m.

September 27: 12 noon, convulsions and death. *Autopsy:* Congestion of the lungs; no other lesions.

Rabbit 423. White, male. Weight, 1,205 grams. Diet, oats.

September 26: 2 p. m., 18 cc 2 per cent caffein administered by mouth; 4 p. m., reflexes increased, no tetanus; 6 p. m., condition unchanged since 4 p. m.

September 27: 9 a. m., found dead. *Autopsy:* Lungs liver, and kidneys congested; other organs normal.

Rabbit 422. White, male. Weight, 1,440 grams. Diet, oats.

September 26: 2 p. m., 21 cc 2 per cent caffein (291 mg per kilo) given by mouth; reflexes increased at 4 p. m.

September 27: 3 p. m., alive, no symptoms; 4 p. m., convulsions with recovery, this was soon followed by a violent attack of tetanus, which lasted about one minute and was succeeded by paralysis; rabbit died at 4.30. *Autopsy:* Liver slightly congested; a small portion of the intestine showed congestion and edema; other organs normal.

A study of these experiments shows also considerable variation in the toxicity of caffein when given by mouth. In some cases a dose of 300 mg per kilo, and even less, caused death, as in rabbits 423 and 424. In other rabbits, however, approximately the same doses of caffein produced increased reflexes only. The same symptoms were produced in Nos. 248 and 249 after the administration of 325–330 mg of caffein per kilo, while another rabbit (No. 239) survived a dose of 363 mg per kilo. That this is exceptional, however, appears from the result of the following experiments on rabbits Nos. 419, 420, and 421, all of which died after receiving 350 mg of caffein per kilo, and rabbits 267 and 268, to which doses of 363 and 342 mg, respectively, per kilo proved fatal. It will be observed further that the gray rabbits are more resistant to caffein than the white animals, as 350 mg per kilo was the smallest fatal dose for rabbits 419, 420, and 421, all of which were gray rabbits, while a dose of 290 mg per kilo was fatal for some of the white rabbits. Again, it will be noticed that of the two gray rabbits, Nos. 254 and 239, which received the largest doses in these experiments, namely, 369 and 363 mg, respectively, one survived. The largest doses given to the white rabbits were 363 and 342 mg caffein per kilo. Both of these died from the effects of the drug. It may be concluded, therefore, that the minimum toxic dose for the gray rabbit is about 325 mg of caffein per kilo, and the minimum fatal dose is at least 350 mg per kilo. It is to be remarked in this connection that post-mortem examination showed extensive coccidiosis in rabbit 420 and fatty liver in No. 421, while the macroscopical examination of the organs of Nos. 424 and 423 failed to show the presence of such abnormalities. Since, as was observed in the section on subcutaneous injection and elsewhere in this investigation, pathological changes are apt to decrease the resistance to caffein, it is quite possible that 350 mg per kilo is not the minimum fatal dose for the normal rabbit. Indeed, the experiment on rabbit 239 lends support to this view, thus furnishing additional evidence of difference in the resistance to caffein in the two varieties of rabbits.

TABLE 2.—*Administration of caffein by mouth.*

SERIES A.

Rabbit No.	Weight.	Caffein per kilo.	Symptoms.	Duration of life.	Diet.	Remarks.
	Grams.	*Mg.*				
248	1,170	330	3 hours 10 minutes	Survived	Oats	Gray.
241	1,380	290	2 hours 15 minutes	do	do	White male.
249	890	325	3 hours	do	do	Gray male.
SERIES B.						
239	935	363		Survived	Oats	Gray male.
254	975	369		About 2 days	do	Gray female.
267	1,050	342	50 minutes	1 hour 4 minutes	do	White.
268	1,100	363		About 3 hours	do	Do.
419	1,600	350	6 hours	Less than 24 hours	do	Gray male.
420	1,250	352	1 hour 35 minutes	2 hours	do	Do.
421	1,485	351	6 hours	Less than 24 hours	do	Do.
424	1,295	293	2 hours	22 hours	do	White male.
423	1,205	300	do	Less than 19 hours	do	Do.
422	1,440	291	do	2½ hours	do	Do.

INJECTION INTO THE PERITONEAL CAVITY.

In a number of experiments caffein was introduced into the peritoneal cavity. Rabbits of different varieties receiving a diet of oats or carrots were employed for this purpose; food and water were given ad libitum. The minimum doses required to induce symptoms or cause death in these animals were determined; tests with caffein were also made on young rabbits in order to study the influence of age on the resistance to this substance. The results are shown in the following experiments:

SERIES A.

In this series large doses were administered, approximating 0.3 gram per kilo.

Rabbit 71. Gray female. Weight, 1,659 grams.

January 20: 2.20 p. m., 25 cc aqueous solution 2 per cent caffein (300 mg per kilo) were injected into the peritoneal cavity; 3.45 p. m., when doors of cage were opened rabbit had spasm of short duration.

January 21: Rabbit found dead.

Rabbit 72. Gray and white. Weight, 1,402 grams.

January 21: 11.17 a. m., 20.2 cc (aqueous solution) of 2 per cent caffein (300 mg per kilo) injected into peritoneal cavity from burette; 11.25 a. m., paralysis; 11.30 a. m., rabbit had convulsion when picked up from the floor, followed by several spasms later; 11.35 a. m., typical tetanus; 12.30 noon, found dead.

Rabbit 61. Black female. Weight, 2,143 grams.

January 19: 40 cc 2 per cent caffein, aqueous solution (300 mg per kilo), injected into peritoneal cavity from burette; tetanus when about 30 cc were injected; when removed from holder, repeated and violent convulsions, terminating in death.

SERIES B.

The object of these experiments was to determine the minimum lethal dose; 0.2 to 0.15 gram of caffein per kilo was injected into the rabbits of this series.

Rabbit 69. White female. Weight, 1,714 grams.

January 20: 10.15 a. m., 6 cc 2 per cent caffein, aqueous solution, injected into peritoneal cavity. No symptoms, under observation for 45 minutes, rabbit defecated rather copiously; feces were soft; 11 a. m., 6 cc 2 per cent caffein, aqueous solution, injected into peritoneal cavity, no symptoms, under observation for 40 minutes; 11.40, 6 cc 2 per cent caffein injected into peritoneal cavity; 11.45, rabbit is restless, reflexes increased.

Rabbit 70. Gray and white female. Weight, 1,487 grams.

January 20: 1.30 p. m., 15 cc 2 per cent aqueous solution of caffein (0.2 gram per kilo) injected into the peritoneal cavity; 2.20 p. m., no symptoms.

January 30: About 2 p. m. rabbit died.

February 1: *Autopsy:* Cirrhosis of the liver; enteritis of small intestines; stomach and kidneys normal.

Rabbit 93. Maltese, male. Weight, 1,197 grams.

March 2: 11.30 a. m., 12 cc of 2 per cent caffein (200 mg per kilo) injected into peritoneal cavity; 11.35 a. m., while being released from holder, tetanus occurred, repeated attacks later, clonic convulsions with tonic rigidity of posterior extremities during the attacks as well as during intervals, anterior extremities were relaxed during the intervals between the attacks, opisthotonos of cervical region but kyphosis in lumbar region were observed, no salivation nor dilatation of the pupils; 2 p. m., rabbit died.

Rabbit 92. Yellow female. Weight, 1,388 grams.

February 25, 4.15 p. m., 14 cc 2 per cent caffein (0.2 gram per kilo) injected into peritoneal cavity; 4.20 p. m., restlessness and increased reflexes, rabbit found stretched out in cage, but raised himself on his legs again; 4.45, general tremor when touched.

February 26: 9 a. m., rabbit found dead. *Autopsy:* Hemorrhage into abdominal muscles at site of injection; hemorrhage also in walls of stomach opposite similar spot in abdominal wall.

Rabbit 309. Belgian hare, female. Weight, 1,500 grams. Diet, oats.

March 2: 2.05 p. m., 2 per cent caffein solution (0.2 gram per kilo) injected into peritoneal cavity; 2.25 p. m., found dead, no urine found in bladder.

Rabbit 307. Belgian hare, female. Weight, 1,320 grams. Diet, oats.

March 2: 12 noon, urine obtained from bladder, clear amber colored, no albumin, no reduction; 12.06 p. m., 10 cc of 2 per cent caffein (0.151 gram per kilo) injected into peritoneal cavity; 1.30 p. m., rabbit placed on floor, runs around but anterior and posterior extremities soon extended, in tonic condition; 2.25 p. m., reflexes increased, paralysis of extremities, dyspnœa; 4.50 p. m., about 100 cc urine collected, no albumin, reduction of Fehling's solution moderate.

March 3: 9.30 a. m., posterior extremities extended and rigid, anterior extremities paralyzed, respiration less frequent and deeper than normal. Rabbit died at 11.50 a. m.; urine collected since 4.50 p. m. previous day gave very heavy reduction of Fehling's solution. *Autopsy:* Animal in good condition; in the left axillary region was observed a hemorrhage into the subcutaneous and muscular tissue of that region. The ventral portion of the large colon, in contact with the ventral abdominal wall, showed a hemorrhagic area about one-half inch in length, such as might be produced by a puncture or bruise of the colon through the abdominal wall. A small portion of the small intestine adjacent to the colon was affected in a similar manner. All internal organs were apparently normal.

Rabbit 308. Belgian hare, female. Weight, 1,350 grams. Diet, oats.

March 2: 11.45 a. m., urine obtained from bladder, no albumen, no reduction; 11.50 a. m., 10 cc 2 per cent caffein (0.15 gram per kilo) injected into peritoneal cavity; 1.30 p. m., not very active, no abnormal symptoms otherwise; 3. 30 p. m., rabbit looked depressed, made very little attempt to move about, remained in one position most of the time when placed on floor; 4.30 p. m., 180 cc urine collected, no albumen. reduction of Fehling's solution moderate.

March 3: 9.30 a. m., rabbit looks normal, is able to walk but is easily fatigued when made to walk about or when placed on his side, followed by paralysis of anterior extremities, posterior extremities apparently normal, about 90 cc of urine collected at noon was free from albumen, did not reduce Fehling's solution.

March 4: 11 a. m., lying on his side in cage, anterior extremities limp, posterior extremities extended and rigid, is in dying condition.

March 5: 9 a. m., found dead. *Autopsy:* Liver engorged; spleen congested, but not enlarged; kidneys, some congestion in cortex; stomach filled, mucosa thickened and easily pulled off; petechial hemorrhages on serosa of colon.

SERIES C.

The experiments of this series were made to determine the minimum toxic dose.

Rabbit 295. Belgian hare, female. Weight, 1,205 grams. Diet, carrots.

March 1: 10.40 a. m., 6 cc 2 per cent caffein (0.1 gram per kilo) injected into peritoneal cavity; about 2 cc of urine obtained before injecting caffein; 2 p. m, 100 cc urine, bloody in appearance, collected, a moderate quantity of albumen present, no reduction; 3.40 p. m., no symptoms.

March 4: 2 p. m., rabbit looks well.

Rabbit 293. Belgian hare, female. Weight, 1,605 grams. Diet, carrots.

March 1: Urine from bladder clear, alkaline; 11.55 a. m., 8 cc 2 per cent caffein (0.1 gram per kilo) injected into peritoneal cavity; 3 p. m., 90 cc urine normal in color collected, no albumen, no reduction; 3.40 p. m., no symptoms.

March 4: 1.15 p. m., rabbit looks normal.

Rabbit 292. Belgian hare, male. Weight, 1,595 grams Diet, carrots.

March 1: 10.10 a. m., 8 cc 2 per cent caffein (0.1 gram per kilo) solution injected into peritoneal cavity; 10.40 a. m., rabbit urinated, reflexes increased, but no other symptoms; 10.50 a. m., no urine obtained from bladder; 2 p. m., 105 cc of clear pale urine collected; no albumen, no reduction; 3.40 p. m., no symptoms.

March 4: 2 p. m., rabbit looks well, urine collected, did not contain sugar.

Rabbit 298. Belgian hare, female. Weight, 1,205 grams. Diet, carrots.

March 1: 4.06 p. m., 7.5 cc 2 per cent caffein solution (0.125 gram per kilo) injected into peritoneal cavity, urine obtained from bladder immediately after injection, no albumen, no reduction; 5.30 p. m., reflexes increased, rabbit was able to run around, but became paralyzed soon; 5.40 p. m., rabbit is again able to run around.

March 3: 10 a. m., anterior extremities paralyzed, is able to use posterior extremities.

March 4: 1 p. m., rabbit looks normal.

Rabbit 223. Belgian hare, male. Weight, 1,165 grams. Diet, carrots.

March 1: 3.50 p. m., urine obtained from bladder clear, amber colored, no albumen, no sugar; 3.52 p. m., 7.5 cc 2 per cent caffein (125 mg per kilo) injected into peritoneal cavity; 5.40 p. m., rabbit makes little attempt to run when put on the floor, weakness of extremities marked.

March 4: 1.15 p. m., rabbit normal.

SERIES D.

The object of the experiments of this series was to study the effect of age on the resistance to caffein. Half-grown rabbits were, therefore, used in the following experiments.

Rabbit 310. Belgian hare, female. Weight, 880 grams. Diet, oats.

March 2: 3.25 p. m., 9 cc 2 per cent caffein (0.2 gram per kilo) injected into peritoneal cavity.

March 3: 9.30 a. m., no symptoms, rabbit looks normal.

March 4: 11 a. m., posterior extremities abducted, walked when placed on the floor, made no attempt to change attitude when placed on its side, remained some time in this position.

March 5: 9 a. m., found dead. *Autopsy:* Liver showed areas of degeneration; kidneys congestion and petechial hemorrhage on cortex; small and large intestines, inflammation marked; bladder distended.

Rabbit 75. Gray and white, female. Weight, 842 grams.

January 25: 3 p. m., 8.5 cc 2 per cent caffein solution (0.2 gram per kilo) injected into peritoneal cavity; 3.15 p. m., anterior extremities weak and reflexes increased.

January 27: Rabbit paralyzed but is able to turn over when placed on back.

Rabbit 74. Gray and white, female. Weight, 692 grams.

January 25: 3 p. m., 7 cc 2 per cent caffein (0.2 gram per kilo) solution injected into peritoneal cavity; 3.15 p. m., reflexes increased and anterior extremities paralyzed.

January 27: Rabbit recovered and is able to walk about in the room.

Rabbit 312, maltese, female. Weight, 740 grams. *Diet, oats.*

March 3: 11.47 a. m., urine obtained from bladder, appearance normal, no albumen, no reduction of Fehling's solution; 11.50 a. m., 7.5 cc 2 per cent caffein (0.2 gram per kilo) injected into peritoneal cavity; 2.30 p. m. anterior extremities paralyzed, posterior extremities rigid and extended; 5 p. m. (about), rabbit died.

Rabbit 311. Belgian hare, female. Weight, 650 grams. Diet, oats.

March 3: 11.26 a. m., urine obtained from bladder normal in appearance, albumen considerable, reduction of Fehling's solution none; 11.27 a. m., 6 cc 2 per cent caffein solution (0.2 gram per kilo) injected into peritoneal cavity; 2.30 p. m., rabbit seemed to be normal, no symptoms had developed; urine collected contained a large amount of sugar, reduction was very heavy, but no albumen was found.

March 4: 11 a. m., condition good, moves about when put on floor; gait, normal.

Rabbit 78. Yellow and white. Weight 659 grams.

January 26: 1.30 p. m., 8.5 cc 2 per cent caffein (250 mg per kilo) injected into peritoneal cavity, under observation the rest of the afternoon, no symptoms.

January 27: 4 p. m., no symptoms developed.

Rabbit 317. Belgian hare, female. Weight 635 grams. Diet oats.

March 15: 10.35 a. m., 8 cc 2 per cent caffein (0. 252 gram per kilo) injected into peritoneal cavity; 12 noon, marked abduction of hind legs, was unable to walk after a little exertion, rabbit died between 12.30 and 12.50 p. m. *Autopsy.* Right lung hepatized and showed adhesions to costal and mediastinal pleura; liver studded with nodules of coccidiosis; spleen congested; stomach filled, mucosa normal; intestines injected; colon hemorrhagic on serosa in ventral region, near point of injection; kidneys normal.

Rabbit 323. White, female. Weight 820 grams. Diet oats.

March 15: 10.45 a. m., 10 cc 2 per cent caffein (250 mg per kilo) injected into peritoneal cavity; 12 noon, reflexes increased, hind legs abducted but is able to walk, symptoms are mild; 1.40 p. m., tremors, weakness, and abduction of head and legs much more marked than at 12 noon.

March 16: Condition good.

March 17: Condition good, recovery apparently complete.

Since the experiments of Series A, which were intended as preliminary tests, have shown that 0.3 gram of caffein per kilo when introduced into the peritoneal cavity is rapidly absorbed and is fatal, much smaller doses were employed in subsequent trials with the drug. This is shown in series B, which may be divided into two groups. Group I, consisting of rabbits 69, 70, 92, 93, and 309, which received 0.2 gram of caffein per kilo, and Group II, Nos. 307 and 308, into which 0.15 gram of caffein per kilo was injected. Three rabbits of Group I (Nos. 92, 93, 309) died from the effects of caffein; rabbit 309 twenty minutes after injection, and rabbits Nos. 92 and 93, twenty hours and two and one-half hours, respectively, after the administration of caffein. In both of these rabbits symptoms appeared within five minutes after the injections were made. Rabbits 69 and 70, it will be noticed, survived the same amount of caffein in proportion to body weight as was given to the other members of this group. Increased peristalsis and the distribution of the dose may account for the greater resistance of rabbit No. 69. The case of rabbit No. 70 is evidently one of exceptional resistance to caffein, since both the rabbits of Group II died from the effects of a much smaller dose, namely, 0.15 gram of caffein per kilo. Moreover, macroscopical examination at the autopsy of Nos. 307 and 308 failed to show any lesions which might tend to lessen the resistance to caffein.

That a dose of 0.15 gram per kilo is therefore in all probability the minimum fatal dose for the rabbit when injected into the peritoneal cavity appears from the results of the experiments in series C, in which smaller doses, 0.125 gram of caffein per kilo caused mild symptoms only, while 0.1 gram per kilo rarely induced any symptoms. It may be remarked that the rabbits of series C were fed carrots while rabbits Nos. 307 and 308 received oats. Their resistance to caffein may be different, but, as was pointed out in the earlier part of this investigation, diet does not seem to influence the toxicity of the single dose of caffein. Doses of 150 and of 100 to 125 mg per kilo, when injected into the peritoneal cavity, may be considered, respectively, as the minimum fatal and minimum toxic doses for the gray rabbit. Analysis of the experiments in series D shows much greater resistance to caffein than in the other rabbits which received it intraperitoneally. Thus, after the administration of 0.2 gram per kilo to each of five rabbits, no effect was observed in two cases (Nos. 310, 311); while in two others (Nos. 74, 75) symptoms developed, but they survived. Only one rabbit, No. 312, died from the effects of this dose; the autopsy showed the presence of degeneration of the liver and petechial hemorrhages on the cortex of the kidneys in the case of No. 310, which was probably the cause of death rather than the caffein.

Two decigrams of caffein can not be considered, therefore, the fatal dose for rabbits. This is further corroborated by the results obtained in experiments with larger doses. Rabbit 78, which received 257 mg per kilo, failed to show any symptoms. The same amount in proportion to body weight in No. 323 caused mild symptoms only, while the rapid death of rabbit No. 317 after the same dose of caffein may be explained by the lesion found at autopsy, thus affording additional evidence that disease may decrease the resistance to caffein. It will be observed that all the members of this series were young rabbits and, as will be shown later, young animals of other species are likewise more resistant to caffein than adult animals. Similar results were obtained by von Anrep, who observed that atropin is less toxic in young than in full-grown animals.

Observations were also made on the diuretic effect of caffein when injected into the peritoneal cavity. The results shown in the following table indicates the stimulating effect on renal secretion whether the diet consisted of oats or of carrots. The urine of some rabbits contained moderate amounts of sugar after from 0.2 to 0.15 gram of caffein per kilo was given; albumen was observed in one case, but in none of the others. In rabbit No. 311 albumin was found before the injection of caffein, but none in the urine which was collected three hours after caffein was injected.

Effect of caffein on renal secretion.

No.	Weight.	Caffein per kilo.	Urine.	Time.	Diet.
	Grams.	*Gram.*	*cc.*		
307	1,320	0.150	100	4.5 hours	Oats.
308	1,305	.150	180	do	Do.
295	1,205	.100	100	2 hours 20 minutes.	Carrots.
293	1,605	.100	90	3 hours	Do.
292	1,595	.100	105	4 hours	Do.

NOTE.—The amount of urine secreted in three hours by control rabbits, on a carrot diet, varied between 35 and 50 cc, the average weight of the animals being a little above 1,600 grams. The secretion of urine on an oat diet was much less for an equal period of time.

TABLE 3.—*Intraperitoneal injections.*

No.	Weight.	Caffein per kilo.	Time of appearance of symptoms.	Duration of life.	Remarks.
			SERIES A.		
	Grams.	*Gram.*			
71	1,659	0.3	85 minutes	24 hours	Gray.
61	2,143	.3		At the end of injection	Black.
72	1,402	.3	8 minutes	1.25 hour	Gray and white.
			SERIES B, GROUP I.		
70	1,487	0.2		10 days	Gray and white.
93	1,492	.2	5 minutes	2.5 hours	Maltese; given second dose after 3 days, died 2.5 hours later.
69	1,492	.2	About 5 minutes	Survived	White.
92	1,388	.2	5 minutes	24 hours	Yellow.
309	1,500	.2		20 minutes	Belgian; oats.
			SERIES B, GROUP II.		
306	1,350	0.15	3 hours and 40 minutes.	About 2.5 days	Belgian; oats.
307	1,320	.15	1 hour 24 minutes	24 hours	Do.
			SERIES C.		
223	1,165	0.125	2 hours	Survived	Belgian; carrots.
293	1,605	.1		do	Do.
295	1,205	.1		do	Do.
292	1,595	.1		do	Do.
298	1,205	.125	1.5 hours	do	Do.
			SERIES D.		
310	880	0.2	2 days [1]	About 2.5 days [1]	Belgian; oats.
311	650	.2		Survived	Do.
312	740	.2	40 minutes	4.5 hours	Maltese; oats.
78	659	.257	15 minutes	Survived	Yellow and white; oats.
75	842	.2	do	do	Gray and white.
74	092	.2	do	do	
317	635	.252	1 hour 25 minutes	About 2 hours	Belgian; oats.
323	820	.25	1 hour 15 minutes	Survived	White; oats.

[1] Not due to caffein.

INTRAMUSCULAR INJECTION.

Well-fed rabbits, which received a diet exclusively of oats, were used for these experiments. The injections were made into the lumbar or into the gluteal muscles.

SERIES A.

In this series the caffein was injected into the gluteal muscles.

Rabbit 284. Brown and white, female. Weight, 1,100 grams.

December 14: 2 p. m., 11 cc 2 per cent caffein injected into the gluteal muscles (0.2 gram per kilo), under observation until 5 p. m., had frequent convulsions; at 5 p. m. in a comatose condition. Rabbit was found dead the next morning.

Rabbit 286, white and black, female. Weight, 1,315 grams.

December 15: 2.30 p. m., 13 cc 2 per cent caffein injected into the gluteal muscles (0.1977 gram per kilo), tremors and increased reflexes observed during the next two hours, but no other symptoms.

December 17: Rabbit alive.

Rabbit 285, yellow and white, female. Weight, 1,385 grams.
December 14: 10.15 a. m., 14 cc 2 per cent caffein injected into the gluteal muscles (0.2 gram per kilo), general tremors, but no convulsions observed. Rabbit survived.
December 17: Rabbit still alive.

Rabbit 287. Belgian hare, female. Weight, 1,140 grams.
December 15: 2.15 p. m., 11 cc of 2 per cent caffein injected into the gluteal muscles; 2.30 p. m., tonic contractions of posterior limbs. Paralysis and death at 2.40 p. m.

SERIES B.

In series B the caffein was injected into the lumbar muscles.

Rabbit 307. Belgian hare, female. Weight, 1,175 grams.
February 16: 11.05 a. m., 8 cc 2 per cent caffein injected (0.136 gram per kilo) into the lumbar muscles; under observation until 4 p. m., no symptoms; 4 p. m., allowed to walk on the floor; after walking a short distance loss of coordination and paralysis of posterior extremities; 5.20 p. m., found dead.

Rabbit 306. Belgian hare, female. Weight, 1,860 grams.
February 16: 11 a. m., 12.5 cc 2 per cent caffein injected into the lumbar muscles; 12 noon, no symptoms; 2 p. m., walked about 10 feet, exhaustion and paralysis; 3 p. m. found dead.

Rabbit 181. Belgian hare. Weight, 1,230 grams. (Was experimented on some time previously.)
February 16: 10.55 a. m., 8 cc 2 per cent caffein injected into the lumbar muscles; (0.130 gram per kilo); 12 noon, no symptoms; 2 p. m., no symptoms; 3 p. m., put on the floor, walked about 10 feet and was exhausted, posterior extremities paralyzed; 4 p. m., found dead.

SERIES C.

In the fall of the same year additional experiments were carried out with doses ranging from 100 to 200 milligrams of caffein per kilo, which were injected into the lumbar muscles. The results are given in the following abbreviated protocols:

Rabbit 425. Belgian hare. Weight 1,520 grams.
September 27: 10.30 a. m., 7.5 cc 2 per cent caffein injected into the lumbar muscles; 2 p. m., reflexes increased.
September 28: Rabbit normal.
October 5: Weight, 1,620 grams; 2.50 p. m., 10 cc 2 per cent caffein injected into lumbar muscles; 3.05 p. m., reflexes increased.
October 13: Weight, 1,520 grams; 10.30 a. m., 10 cc 2 per cent caffein (131 mg per kilo) injected; 11 a. m., no symptoms; 11.30 a. m., reflexes much increased.
October 14: Alive, no symptoms.

Rabbit 426. Belgian hare, female. Weight, 1,425 grams.
September 27: 7 cc 2 per cent caffein injected into the lumbar muscles at 10.30 a. m.; 2 p. m., reflexes increased.
September 28: Rabbit normal.
October 5: Weight, 1,425 grams; 2.55 p. m., 9 cc 2 per cent caffein injected into lumbar muscles; 3.05 p. m., reflexes increased.
October 13: Weight, 1,405 grams; 10.30 a. m., 10 cc 2 per cent caffein (142 mg per kilo) injected; 11 a. m., no symptoms; 11.30 a. m., reflexes increased.
October 14: Rabbit alive, no symptoms.

Rabbit 427. Belgian hare, female. Weight, 1,780 grams.
September 27: 9 cc 2 per cent caffein injected into the lumbar muscles; 2 p. m., reflexes increased.
September 28: Rabbit normal.
October 5: Weight, 1, 850 grams; 3 p. m., 11.5 cc 2 per cent caffein injected into lumbar muscles; 3.10 p. m., reflexes increased.
October 13: Weight, 1,830 grams; 10.40 a. m., 14 cc 2 per cent caffein (153 mg per kilo) injected into lumbar muscles; 11 a. m., no symptoms; 11.30 a. m., reflexes increased.
October 14: Rabbit alive, no symptoms.

Rabbit 453. Belgian hare, male. Weight, 1,160 grams.
October 12: 3.45 p. m., 11.5 cc 2 per cent caffein in aqueous solution injected into lumbar muscles; 4.15 p. m., reflexes increased; 4.30 p. m., paralyzed.

October 13: 9 a. m., found dead. *Autopsy:* Gastric mucosa hemorrhagic; liver darkened; other organs normal.

Rabbit 455. Belgian hare, gray, female. Weight, 1,185 grams.

October 12: 3.30 p. m., 11.5 cc 2 per cent caffein injected into the lumbar muscles; 4 p. m., reflexes increased.

October 13: Rabbit weighed 1,070 grams, no symptom of caffein poisoning, reflexes normal; 10.30 a. m., 10 cc 2 per cent caffein injected into the lumbar muscles; 11.30 a. m., jumped off the table, had attack of convulsions and died. *Autopsy:* Findings same as in No. 453.

Rabbit 428. Belgian hare, gray, male. Weight, 1,650 grams.

October 5: 4 p. m., 14.8 cc 2 per cent caffein (0.18 gram per kilo) injected into the lumbar muscles.

October 6: Found dead.

Rabbit 429. Belgian hare, male. Weight, 1,340 grams.

October 5: 4 p. m., 13.5 cc 2 per cent caffein (0.2 gram per kilo) injected into lumbar muscles.

October 8: Rabbit found dead.

SERIES D.

Further experiments making injections into both the lumbar and the gluteal muscles, were made in this series.

Rabbit 577. Gray male. Weight, 1,380 grams.

February 14: 3 p. m. 14 cc 2 per cent caffein injected into the gluteal muscles of the right side; 3.10 p. m., restless, jumped off the table and walked about, reflexes increased; 3.45 p. m., passed 30 cc clear, straw-colored urine; 4.45 p. m., allowed to walk about, ran across the room, about 20 feet, looked tired, stretched himself out on the floor, then raised himself and walked about showing no disturbance of gait.

February 15: 9 a. m., found dead.

Rabbit 578. Gray, female. Weight, 1,670 grams.

February 14: 3.05 p. m., 18 cc 2 per cent caffein solution injected into the gluteal muscles of the right side; 3.15 reflexes increased, but not restless; 5 p. m., allowed to walk about, no symptoms observed.

February 15: Found dead.

Rabbit 579. White and gray, male. Weight, 1,490 grams.

February 14: 3.15 p. m., 15 cc 2 per cent caffein solution injected into the gluteal muscles of the right side, put in cage; 3.30 p. m., reflexes increased; 5 p. m., taken out of cage and allowed to walk across the room, no special symptoms noticed.

February 23: Still alive.

Rabbit 580. Gray male. Weight, 1,510 grams.

February 14: 3.35 p. m., 15 cc 2 per cent caffein solution injected into lumbar muscles.

February 23: Still alive, in good condition.

Rabbit 581. Gray female. Weight, 1,680 grams.

February 14: 3.45 p. m., 17 cc 2 per cent caffein solution injected into the lumbar muscles of the right side; 4 p. m., reflexes increased; 4.15 p. m., jumped off the table and had wild convulsions, became very restless, walked about the laboratory; 4.25 p. m., had convulsions occasionally; 4.30 p. m., extremities extended and quite rigid; 4.35 p. m., convulsions and death.

Rabbit 582. Gray male. Weight, 1,870 grams.

February 14: 4.15 p. m., 18 cc 2 per cent caffein solution injected into the lumbar muscles of right side; 5 p. m., reflexes increased; walked about in the room, then rested; 5.15 p. m., had short spasm when handled.

February 23: Alive; good condition.

The data presented in these experiments show that the toxicity of caffein when injected into the muscles of the lumbar regions is the same as when injected into the gluteal muscles. The rabbits of series A received approximately 0.2 gram caffein per kilo and two died as a result of this treatment. The other two survived but symptoms of caffein intoxication were observed.

In series B smaller doses proved fatal, from which it would appear that caffein is more toxic when injected into the lumbar muscles. Further observations, however, failed to corroborate the results obtained in this series. Thus, in series C, 130 to 150 mg of caffein per kilo injected into the lumbar muscles produced mild symptoms only. Experiments with larger doses showed that 0.180 gram caffein per kilo may cause death. It will be noticed, on the other hand, that rabbit No. 455 survived a dose of 0.2 gram per kilo. New experiments were therefore carried out in which the same amounts of caffein in proportion to the weight of the animals were injected into the lumbar muscles as into the gluteal muscles. As shown in the experiments of series D, one rabbit (No. 581) died shortly after caffein was injected into the lumbar muscles; two recovered. Two of the three which received injections into the gluteal muscles were found dead the next day; one recovered. Post-mortem examination failed to indicate the presence of any abnormalities. The rate of absorption of caffein from the gluteal and from the lumbar muscles seems to be, therefore, the same, or not to differ very much. The observations of Auer and Meltzer[7] are of interest in this connection. According to their investigations adrenalin is more rapidly absorbed from the lumbar than from the gluteal muscles. This is in all probability due to the greater delicacy of the test they employed (since they judged the rate of absorption by the effect of adrenalin on blood pressure) as well as to the much greater activity of the substance.

TABLE 4.—*Intramuscular injections.*

SERIES A.

No.	Weight.	Caffein per kilo.	Symptoms after—	Duration of life.	Site of injection.	Remarks.
	Grams.	*Gram.*				
284	1,100	0.200	3 hours	Less than 20 hours	Gluteal	White and brown female.
286	1,315	.1977	2 hours	Survived	do	White and black female.
285	1,385	.200	Present	do	do	Yellow and white female.
287	1,140	.210	15 minutes	25 minutes	do	Gray female.

SERIES B.

307	1,175	0.136	5 hours	6 hours, 20 minutes	Lumbar	Gray female.
306	1,860	.134	3 hours	4 hours	do	Do.
181	1,230	.130	4 hours	5 hours	do	Gray.

SERIES C.

425	1,520	0.131	1 hour	Survived	Lumbar	Gray.
426	1,405	.142	30 minutes	do	do	Gray female.
427	1,830	.153	50 minutes	do	do	Do.
453	1,160	.200	30 minutes	Less than 20 hours	do	Gray male.
455	1,185	.200	do	Survived	do	Gray female.
428	1,650	.180		Less than 20 hours	do	Gray male.
429	1,340	.200		do	do	Do.

SERIES D.

577	1,380	0.200	10 minutes	Less than 18 hours	Gluteal	Gray male.
578	1,670	.210	do	do	do	Do.
579	1,490	.200	15 minutes	Survived	do	White and gray male.
580	1,510	.200		do	Lumbar	Gray male.
581	1,680	.200	15 minutes	50 minutes	do	Do.
582	1,870	.192	45 minutes	Survived	do	Do.

Examination of Table 4 shows that 14 rabbits received from 180 to 210 mg caffein per kilo. The appearance of symptoms in these rabbits varied considerably. In some increased reflexes could be noticed in 10 to 15 minutes after the injection of caffein; in others it was delayed 2 or 3 hours. It might be added that the onset of symptoms occurred in many cases very soon after the administration of the drug—on an average about 10 to 30 minutes after the drug was injected. After smaller doses were administered by injection into the lumbar muscles the appearance of symptoms was delayed several hours in some cases. The duration of life in these 14 rabbits varied considerably. Eight of them died within 1 to 20 hours; six survived. About 0.2 gram caffein per kilo may be regarded as the minimum fatal dose, while the minimum toxic dose is somewhere between 130 and 150 mg per kilo.

INTRAVENOUS INJECTION.

These experiments were carried out on well-fed, full-grown gray rabbits. The diet for several days preceding the experiments consisted of oats or carrots, which were given ad libitum. The injections were made into the ear veins from a burette or by means of a syringe, the temperature of the caffein solution being about 40° C. Attention was also directed to the effect of the rate of injection and of the concentration on the toxicity. The minimum toxic as well as lethal doses were determined as shown in the following experiments.

SERIES A.

In these experiments the rate of injection was about 1 cc of 2 per cent caffein solution per minute.

Rabbit 194. White, female. Weight, 1,310 grams.

October 19: Injected 7.5 cc 2 per cent solution caffein (115 mg per kilo) into the ear vein. Rabbit showed stiffness; paralysis of extremities appeared soon after.[a] Rabbit survived.

Rabbit 556. Gray, female. Weight, 1,635 grams.

January 31: 2 p. m., 11 cc 2 per cent caffein (134 mg per kilo) injected into ear vein, in about 11 minutes; 2.10 p. m., convulsions, rabbit remained lying on its side; during the rest of the hour it had convulsions occasionally; 3.20 p. m., convulsions and died. Rabbit did not urinate after the injection of caffein.

Rabbit 557. Gray, female. Weight, 1,580 grams.

January 31: 2.30 to 2.37 p. m., 7 cc 2 per cent caffein injected from the burette at the rate of 1 cc per minute; 2.37 p. m., flow of liquid ceased, veins were engorged and bled freely, injection was continued by means of a syringe; 2 cc 2 per cent caffein injected in two minutes; injections discontinued as convulsions appeared; 2.50 p. m., rabbit raised itself but fell over; 3.10 p. m., rabbit assumed normal attitude, walked about the floor without manifesting any signs of the effects of caffein; 4.30 p. m., walked about, gait normal, condition seemed to be good.

February 1: 2 p. m., condition good, appetite good, total amount of caffein injected, 9 cc 2 per cent solution, or 114 mg per kilo.

Rabbit 558. Gray, female. Weight, 1,590 grams.

January 31: 3 p. m., given 8 cc 2 per cent caffein in eight minutes; 3.10 p. m., violent convulsions; 3.20 p. m., rabbit was stretched out on his abdomen, extremities extended, urinated; 4.30 p. m., looked normal; was able to walk about.

February 1: 2 p. m., condition good, appetite good.

Rabbit 292. Belgian hare, male. Weight, 1,770 grams.

February 18: 4.26 to 4.39 p. m., 12.5 cc warm caffein solution (0.141 gram per kilo) injected into ear vein, convulsion followed when this quantity was injected, tonic rigidity of limbs followed soon after; 4.52 p. m., condition unchanged, rabbit on floor, limbs stretched out, and lying on abdomen.

Rabbit 294. Belgian hare, female. Weight, 1,350 grams. Carrot diet for about 10 days before the experiment.

February 19: 12.20 p. m., 5 cc 2 per cent caffein (74 mg per kilo) injected into ear vein in five minutes, edema of the ear, other ear used, 3.5 cc injected in 10 minutes, repeated convulsions; 1.25 p. m., rabbit still alive, frequent attacks of convulsions; 2.30 p. m., found dead. Total amount injected in 15 minutes, 8.5 cc, or 0.126 gram per kilo.

It will be observed in the preceding experiments that symptoms of severe intoxication were present in all of the six rabbits, but only two of these (Nos. 294 and 556) died from the effects of caffein. Of those which survived, three received doses of

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[a] Time of injection inadvertently omitted, but was probably not slower than in the other cases of this series.

100 to 114 mg caffein per kilo, and another (No. 292) received 141 mg of caffein per kilo. The death of rabbits Nos. 294 and 556 may be regarded therefore as a case of exceptionally low resistance to caffein.

SERIES B.

Doses of 160 to 200 mg caffein per kilo were employed in these experiments. The rate of injection was 1 cc per minute, with the exception of Experiment 254, in which 10.8 cc 2 per cent caffein were introduced in 17 minutes and 25 seconds.

Rabbit 562. Gray female. Weight, 1,650 grams. Diet, oats.

February 1: Injection began at 3 p. m., injected 10 cc in 12 minutes; 3.01 p. m. to 3.09 p. m., 3 cc injected, convulsions; 3.09 p. m. to 3.14 p. m., 3 cc injected, followed by violent convulsions, marked opisthotonos; 4.30 p. m., rabbit died; total quantity injected, 16 cc.

Rabbit 561. Gray female. Weight, 1,450 grams. Diet, oats.

February 1: Injection began at 11.40 a. m.; 11.48, rabbit struggled, 7 cc 2 per cent caffein injected; 11.50, convulsions, 10 cc 2 per cent caffein total amount injected; 11.55 a. m., injections stopped; injections resumed 11.58, violent convulsions, injections discontinued, total quantity received, 14.5 cc 2 per cent caffein solution; 1.30 p. m., found dead, did not urinate, 25 cc urine found in the bladder.

Rabbit 560. Gray male. Weight, 1,620 grams. Diet, oats.

February 1: Injection began 11 a. m.; 11.10 a. m., 7 cc 2 per cent caffein injected, rabbit struggled; 1 cc was injected during the next three minutes, rabbit struggled but there were no convulsions, injection stopped; resumed at 11.15 a. m. and continued 10 minutes, 8 cc 2 per cent caffein introduced during this time; total amount caffein injected, 16 cc; reflexes markedly increased; 12 noon, tetanic convulsions off and on until 2 p. m., then remained stretched out on abdomen, extremities extended.

February 2: 9 a. m., found dead.

Rabbit 559. Gray female. Weight, 1,875 grams. Diet, oats.

January 31: 4 p. m., convulsions after injection of 9 cc 2 per cent caffein in 14 minutes; 4.08 p. m., convulsions after injection of 7 cc caffein in 8 minues; 4.10 to 4.12 p. m., injected 2 cc more, rabbit lying stretched out on abdomen, extremities extended; total amount of caffein injected, 18 cc (190 mg per kilo).

February 1: 2 p. m., condition good, walked about, appetite good, passed 155 cc dark, reddish-brown urine since 5.30 p. m. previous day.

Rabbit 279. Gray and white female. Weight, 1,320 grams.

February 24: 10.09 a. m., 6 cc 2 per cent caffein passed rapidly into jugular vein; 10.15 a. m., involuntary twitching of muscles of legs, but no other symptoms; 10.23 to 10.26, 3 cc of 2 per cent caffein injected; 10.27 to 10.28, 2 cc 2 per cent caffein injected, convulsions; 10.29, convulsions stopped; 10.32, convulsions; 11 a. m., rabbit lying on its side, anterior extremities paralyzed, posterior extremities contracted, no clonic convulsions, breathed deeper and more slowly than normal; 11.10 a. m., rabbit died, had no convulsions immediately before death; amount of caffein injected, 11 cc 2 per cent solution, or 0.166 gram per kilo.

Rabbit 254. Belgian hare, female. Weight, 1,285 grams. Diet, oats.

November 12: 1.30⅓ to 1.47¾ p. m., received 10.8 cc 2 per cent caffein from burette into ear vein, after injection of 6.2 cc dyspnœa, 6.7 cc struggling, convulsions; at 1.50½ p. m., released from holder, paralysis especially marked in the anterior extremities; 1.50 p. m., recovered, survived; total amount injected, 10.8 cc 2 per cent caffein in 17 minutes and 25 seconds, or 0.16 gram caffein per kilo.

Rabbit 255. Belgian hare, male. Weight, 1,105 grams. Diet, oats.

November 12: 2.31¾ to 2.35¼ p. m., received 3.7 cc; from 2.37⅙ to 2.46⅙ p. m., 5 cc injected; after injection of 6.1 cc convulsions followed by dyspnœa, then continuous struggling; when 8.3 cc were injected rabbit had another convulsion; 2.47 p. m., tonic contraction of anterior extremities; amount injected, 8.7 cc (158 mg per kilo) in 15 minutes and 35 seconds.

Rabbit 567. Gray female. Diet, oats.

February 6: Injection began at 4.11 p. m.; 4.18, convulsions after injection of 5 cc 2 per cent caffein; 4.21, convulsion after total injection of 8 cc; 4.24 p. m., injection resumed and 2 cc more introduced; 4.28 p. m., convulsions, injected 2 cc more; total caffein injected, 12 cc, or 162 mg per kilo; 4.40 p. m., rabbit paralyzed in posterior extremities; 5 p. m., found dead.

In the eight experiments comprising series B rabbits Nos. 567, 254, 279, and 255, which may be designated as Group II, received doses of 162, 160, 166, and 158 mg, respectively. Nos. 562, 561, 560, and 559, which may be designated as Group I, received about 200 mg caffein per kilo. In Group II, which received the smaller doses, one (No. 254) survived. This may be regarded as exceptional, since, as was shown in the experiments of the preceding series, even smaller doses may be fatal. About 160 mg per kilo is, therefore, the smallest surely fatal dose. This might be regarded as a contradiction of the results obtained for rabbit No. 559, but it will be noticed that in this case diuresis was very marked. The results of experiments Nos. 294 and 255 are of interest in this connection, since they indicate that a moderate difference in the rate of injection is without any effect on the toxicity of caffein. The greater resistance to caffein of rabbit No. 559 is in all probability due, therefore, to increased diuresis.

SERIES C.

In these experiments the minimum toxic dose was determined. The conditions were the same as in the experiments of the other series.

Rabbit 293. Belgian hare, female. Weight, 1,610 grams. Diet, oats.

February 18: 3.40 to 3.43 p. m., 4 cc 2 per cent warm caffein solution injected into ear vein, convulsions when 3 cc were injected, repeated attacks; 4 p. m., raised itself on legs, but fell over immediately and lay stretched on abdomen.

February 19: 9 a. m., rabbit looked normal, apparently recovered.

Rabbit 227. White male. Weight, 2,320 grams.

October 26: 3.29½ to 3.37½ p. m., injected into ear from burette 6.7 cc 2 per cent caffein, no symptoms; experiment discontinued; survived.

Rabbit 563. Gray female. Weight, 1,650 grams. Diet, oats.

February 6: Injection began at 1.02 p. m., injected 3.5 cc 2 per cent caffein (42 mg per kilo) in four minutes, 0.6 cc more within the next two and one-half minutes, total amount injected 4.1 cc; 1.10 p. m., hypersensitive, some disturbance of muscular coordination; restlessness; 1.35. p m., reflexes decreased, urinated and walked about, gait normal. Under observation for several days; no symptoms noted.

Rabbit 564. Gray female. Weight, 1,515 grams.

February 6: Injection began at 1.26 p. m., 3.5 cc 2 per cent caffein (46 mg per kilo) injected at the rate of 1 cc per minute; 1.30 p. m., reflexes increased; 1.34 p. m., marked paresis of the extremities, rabbit stretched out on abdomen, legs abducted and partly extended, able to hop about but gait disturbed, no untoward symptoms noticed, under observation for several days after experiment.

Rabbit 565. Gray female. Weight, 1,545 grams. Diet, oats.

February 6: Started to inject at 3.40 p. m., received 2.5 cc 2 per cent caffein intravenously in two minutes or 32 mg per kilo, under observation all afternoon, no symptoms.

Rabbit 566. Gray female. Weight, 1,900 grams. Diet oats.

February 6: Injection began at 3.05 p. m., received 3 cc 2 per cent caffein intravenously in three minutes or 31 mg per kilo, no symptoms observed.

These experiments show that a dose of about 50 mg per kilo when injected intravenously produces mild symptoms, such as increased reflexes. In the four experiments with this amount of caffein these effects were observed in each case. In the experiments in which smaller quantities, 30 mg per kilo, were given intravenously there was no manifestation of symptoms. A dose not over 50 mg per kilo may, therefore, be regarded as the minimum toxic dose when injected intravenously under the conditions stated.

SERIES D.

A 0.5 per cent caffein solution was used in these experiments in order to test the effect of concentration on its toxicity; the rate of injection was 1 cc per minute.

Rabbit 569. Gray male. Weight, 1,475 grams. Diet, oats.

February 6: 11.50 a. m. to 12.01 p. m., injected 10 cc 0.5 per cent caffein; 12.03 to 12.12 p. m., injected 10 cc of 0.5 per cent caffein; 12.13 to 12.26 p. m., injected

10 cc of 0.5 per cent caffein, total amount injected, 30 cc; 12.20, passed 35 cc of urine; 12.30, increased reflexes, but no convulsions; 4 p. m., reflexes increased.
February 11: Alive, condition good.

Rabbit 574. Gray female. Weight, 1,555 grams. Diet, oats.
February 8: 10.25 to 10.33 a. m., injected 4 cc of 0.5 per cent caffein in salt solution, injection discontinued for five minutes; 10.38 to 11.10, injected 30 cc, total amount of caffein solution received, 34 cc; 11.55 a. m., very sensitive; reflexes markedly increased.
February 9: Alive, condition good.

Rabbit 571. Gray female. Weight, 1,530 grams. Diet, oats.
February 7: Injection 3.18 to 3.50 p. m., received 30 cc in 32 minutes, not hypersensitive; 3.55, restlessness and weakness of extremities; 4.10 p. m., control of anterior extremities impaired, distinctly paretic but tried to walk about, died the same afternoon.

Rabbit 568. Gray male. Weight, 1,605 grams. Diet, oats.
February 7: Injection 10.53 to 11.01 a. m., injected 10 cc 0.5 per cent caffein; 11.03, injection resumed after two minutes interval; 11.14, received 10 cc 0.5 per cent caffein intravenously in 11 minutes; 11.16, injection resumed; 11.35, received 12 cc 0.5 per cent caffein, total amount of caffein solution received, 32 cc; 12.30 p. m., urinated 14 cc of bloody urine; 12.55 p. m., convulsions and death a few minutes later. Autopsy showed congestion of viscera, but no other lesions.

Rabbit 570. Gray female. Weight, 1,225 grams. Diet, oats.
February 7: 2.06 to 2.35 p. m., injected 24.5 cc 0.5 per cent caffein, reflexes increased but no convulsions, paresis especially marked in the anterior extremities; 3 p. m., passed urine which was normal in appearance, reflexes not increased but rabbit was weak.
February 9: Found dead. *Autopsy:* Liver, spleen, and kidneys congested; large intestines hemorrhagic; omentum congested and showed the presence of small caseous nodules; liver showed adhesion to diaphragm; viscera presented the appearance of intraabdominal infection.

Of the five rabbits of this series three died as a result of the administration of caffein. The other two which survived showed mild symptoms only, such as increased reflexes, but no evidence of severe poisoning such as was observed after the injection of the same doses of caffein in series A when a 2 per cent solution of caffein was injected. Convulsions were noticed in one case only (No. 568); paresis in two cases (Nos. 570 and 571). The nervous symptoms even in this group, therefore, were much milder than in series A. The percentage of death, however, was greater than in series A, in which the concentration of caffein was four times as great. It is quite probable that the strain on the heart due to the sudden increase in volume of the blood and its dilution might be an important factor in increasing the toxicity of caffein. It is conceivable that doses just sufficiently large to depress the normal heart may cause paralysis of an already overstrained organ.

SERIES E.

In the two experiments of this series the rate of injection as a possible factor influencing the toxicity of caffein was tested. A 2 per cent caffein solution was injected at the rate of 1 cc in two and one-half to three minutes.

Rabbit 572. Gray male. Weight, 1,770 grams. Diet, oats.
February 8: Injection began at 3 p. m., discontinued at 3.37 p. m., and resumed at 3.38 p. m.; rabbit was restless; injection finished at 3.52 p. m. Total quantity received, 17.4 cc 2 per cent caffein intravenously in 52 minutes; struggled intermittently during the injection; anterior legs paralyzed.
February 9: Found dead.

Rabbit 573. Gray male. Weight, 1,810 grams. Diet, oats.
February 8: Started to inject at 1.35 and discontinued at 2.27 p. m.; received 18 cc 2 per cent caffein intravenously in 52 minutes; reflexes markedly increased soon after; 2.45, passed bloody urine; 4.30 p. m. reflexes increased; no other symptoms.
February 9: 9 a. m., found dead.

It will be observed that some retardation of the onset of symptoms was caused by slower injection, but the final result was the same as when the injections were made more rapidly. It is quite probable, therefore, that a much slower rate of injection may lessen considerably the toxicity of caffein.

From the results of the experiments by intravenous injection summarized in the table, it appears that the minimum toxic dose for rabbits of a 2 per cent caffein solution, injected at the rate of 1 cc per minute, is about 50 mg per kilo. Twice the dose induces severe symptoms and may be fatal; 160 mg per kilo are surely fatal. If the rate of injection is diminished, the toxicity of caffein is lessened, but this effect is not marked unless the injections are very slow. Dilution of the caffein solution suppresses to some extent the nervous symptoms, but the toxicity, on the contrary, seems to be increased.

TABLE 5.—*Intravenous injections.*

SERIES A.

No.	Weight.	Caffein per kilo.	Symptoms.	Duration of life.	Diet.	Remarks.
	Grams.	*Mg.*				
194	1,310	114	Present...	Survived..................	Oats......	White female.
556	1,635	134	10 minutes	20 minutes................	...do......	Gray female.
557	1,580	114	Present...	Survived..................	...do......	Do.
558	1,590	100	...do......	.....do....................	...do......	Do.
292	1,770	141	...do......	.....do....................	...do......	Do.
194	1,350	126	...do......	10 minutes................	Carrots....	Do.

SERIES B, GROUP I.

562	1,650	200	....	1½ hours..................	Oats......	Gray female.
561	1,450	200	....	.....do....................	...do......	Do.
560	1,620	200	Present...	Less than 24 hours........	...do......	Do.
559	1,875	190	...do......	Survived..................	...do......	Do.

SERIES B, GROUP II.

279	1,320	166	....	1 hour....................	....	Gray and white female.
254	1,285	160	....	Survived..................	Oats......	Gray female.
567	....	162	....	About 45 minutes.........	....	Do.
255	....	158	....	Died......................	....	

SERIES C.

293	1,610	500	Present...	Survived..................	Oats......	Gray female.
227	2,320	570	None......	.....do....................	....	White male.
563	1,650	500	Present...	.....do....................	...do......	Gray female.
564	1,515	460	...do......	.....do....................	....	Do.
565	1,545	320	None......	.....do....................	...do......	Do.
566	1,900	310	...do......	.....do....................	...do......	Do.

SERIES D.

569	1,475	100	Present...	Survived..................	Oats......	Gray male.
574	1,555	112	...do......	.....do....................	...do......	Gray female.
571	1,530	100	...do......	About 2 hours.............	...do......	Do.
568	1,605	100	....	20 minutes................	...do......	Gray male.
570	1,225	100	....	Less than 20 hours.......	...do......	Do.

SERIES E.

572	1,770	200	Present...	About 24 hours...........	Oats......	
573	1,810	200	....	.... do.................	...do......	

SUMMARY.

The results of the experiments on rabbits show considerable variation in the toxicity of the single dose. Individuals differed so widely in their resistance to this drug that the same experiments had to be repeated many times with each method of administration before satisfactory conclusions could be drawn. This is strikingly illustrated in the experiments by intravenous injection in which a dose of nearly 0.2 gram per kilo was not fatal.. Similar instances of exceptional resistance or of sensitiveness to caffein were observed when it was given in other ways. A comparison of the toxicity of caffein administered by different methods in this investigation shows well-marked differences in its activity, although they are not quite so striking as similar experiments with other alkaloids reported by several observers. The toxicity of caffein in these experiments on the rabbit indicates that it is greatest when given by vein and least when given by mouth. The ratio of the minimum toxic doses by these two methods of introduction of caffein was about 7.1; the relation of the minimum fatal dose was about 3.1. The toxicity when given subcutaneously is about 15 to 20 per cent greater than when given by mouth. The difference between the intramuscular and subcutaneous injection is even more marked. The toxicity of caffein when injected into the muscles is about midway between that administered by the subcutaneous and intraperitoneal routes, and is about half that injected intravenously. Meltzer and Auer,[58] who experimented with a number of drugs found that the intramuscular method of administration is as effective as the intravenous, fluorescin forming the only exception according to their observations. In the experiments of Sollman and Brown[81] with ergot, the effect was quite different from those obtained by Meltzer and Auer[58] with the drugs they used. It is quite possible that the result obtained with ergot is merely illustrative of a difference in the behavior of various substances in this regard. This appears probable on account of the difference in the rate of absorption for various substances. Thus, according to Achard, Gaillard, and Ribot (Compt. rend. Soc. biol., 1907, *62*: 90), absorption from the peritoneal cavity varies with the concentration of the solution and the size of the molecule. The smaller the molecule and the greater the concentration the more rapid the absorption. That the rate of absorption from the intramuscular tissues is unequal and varies for different substances appears from the experiments of Meltzer and Auer.[58] The difference was very striking between intramuscular and subcutaneous administration of curara or adrenalin; the results were somewhat different with morphin and with fluorescin. As shown in their protocols, the onset of the symptoms after the intramuscular injection of morphin was sooner than after subcutaneous injection, but in time the difference diminishes and disappears altogether. The absorption of fluorescin is much faster when the intramuscular path is used than when given subcutaneously, but the writers state that the rate falls far behind that of the intravenous administration. The difference in toxicity we observed between feeding by mouth and subcutaneous injection, although distinct, was not very great. It was much less than Maurel[55] obtained with the hydrobromid of caffein in the rabbit. Whether this difference between his results and ours is due to the use of the pure alkaloid in our experiments and the hydrobromid employed by Maurel can not be stated at present with any degree of accuracy. It is hoped that the work in progress in the laboratory will throw some light on the subject in the near future. But Maurel's[56] experiments show that various substances behave differently in this regard. Thus the toxicity of strychnin, he states, is three times as great when given subcutaneously as when given by mouth and six times that of the minimum fatal dose by vein. It may be remarked, however, that examination of his data shows that his doses are much too large for the rabbit. In experiments with other drugs little or no difference between the two modes of administration was noticed. Thus, digitalin was but slightly more active when given subcutaneously than by mouth, while the toxicity of emetin hydrochlorid was just the same, whichever one of these methods of introducing the substance was used. Differences in the toxicity of substances have also been observed between subcutaneous and intravenous modes of administration, but here, too, the differences for various substances were unequal.

EXPERIMENTS ON GUINEA PIGS.

The toxicity of caffein was studied in a large number of individuals. The experiments were conducted on full-grown animals and were carried out at different seasons of the year in a variety of ways. Special attention was given to diet as a possible factor influencing resistance to caffein, and the effect of different modes of administration on toxicity. Some animals were therefore fed oats, some carrots, others received both hay and oats. Caffein was introduced subcutaneously, intraperitoneally, and by mouth.

SUBCUTANEOUS INJECTION.

SERIES A.

Preliminary experiments carried out on three guinea pigs, which received 360, 300, and 290 mg of caffein per kilo subcutaneously have shown that such doses were rapidly fatal. Two of the animals were seized with convulsions half an hour after the introduction of caffein and died during the attack. The other had tetanus two minutes after the injection of caffein. Repeated attacks followed, which terminated in the death of the animal two and a half hours later. The fatal and toxic doses must therefore be considerably under 0.3 gram of caffein per kilo when introduced by this path and smaller doses were therefore injected. The results are shown in the experiments of the next series.

SERIES B.

Experiments with 2 decigrams per kilo constituted this series.

Guinea pig 20. Female. Weight, 497 grams. Diet, oats.

April 2: 5 cc 2 per cent caffein injected subcutaneously at 11.30 a. m.; 1.50 p. m., spasm of short duration. Died at 3 p. m., three and one-half hours after injection.

Guinea pig 38. Brown male. Weight, 570 grams. Diet, carrots and oats week previous to injection.

February 11: 3.50 p. m., 6 cc 2 per cent caffein injected subcutaneously in back (210 mg per kilo); 4.15, reflexes increased, had convulsion of short duration when disturbed; 4.45 p. m., on handling, repeated convulsion and paralysis; 5 p. m., guinea pig lying on his side, respiration difficult and labored.

February 11: 5.05 p. m., guinea pig found dead, 2 hours and 15 minutes after injection.

Guinea pig 37. Male. Weight, 820 grams. Diet, carrots and oats during week preceding the injection of caffein.

February 11: 3.35 p. m., 8.5 cc 2 per cent caffein injected subcutaneously in the back; 5 p. m., pig very sensitive, anterior extremities paralyzed when handled, frequent spasms of posterior extremities, no symptoms noticed before 5 p. m., although watched all the time; 5.05 p. m., guinea pig on his legs and looked normal. No attack on handling.

February 12: 9 a. m., found dead; died within 18 hours.

Guinea pig 13. Female. Weight, 618 grams. Diet, oats.

March 29: 2.45, 6 cc 2 per cent caffein injected subcutaneously (0.194 grams per kilo).

March 30: Died at 4 p. m., 25 hours after injection.

Guinea pig 36. Male. Weight, 850 grams. Fed oats and carrots for one week previous to injection.

February 11: 3.30 p. m., 8.5 cc 2 per cent caffein injected subcutaneously into back; 5 p. m., somewhat more sensitive than normal, no other symptoms, no effect on handling; 5.05 p. m., no symptoms.

February 12: 9 a. m., found dead, about 18 hours after injection.

The results of these experiments, as observed in five guinea pigs, indicate that two decigrams of caffein per kilo of animal produce symptoms within a half to about two and a quarter hours after injection. Death followed in two guinea pigs 70 minutes to 1 hour after the first manifestations of symptoms. Two others died during the night, while one lived 25 hours after the injection of caffein. Even 2 decigrams caffein per kilo weight might therefore be fatal to the guinea pig. Experiments carried out later have shown, however, that the resistance to caffein is appreciably greater in some guinea pigs. This is indicated by the following experiments, in which doses of 0.2 to 0.24 gram caffein per kilo were administered by the same path.

SERIES C.

Guinea pig 66. Yellow and dark brown male. Weight, 510 grams. Diet, oats.

October 4: 5 cc 2 per cent caffein (0.2 gram per kilo) injected subcutaneously in the back at 3 p. m.; 5 p. m., no symptoms.

October 5: 9 a. m., alive; condition good.

October 9: Found dead. *Autopsy:* Congestion of liver, kidney, and small intestine.

Guinea pig 65. White and black male. Weight, 510 grams. Diet, oats.

October 4: 5 cc 2 per cent caffein (0.2 gram per kilo) injected subcutaneously in the back at 3 p. m.; 5 p. m., no symptoms.

October 5: 9 a. m., condition good.

Guinea pig 60. White and gray female. Weight, 320 grams. Diet, oats.

October 3: 2.25 p. m., 3.5 cc 2 per cent caffein ((0.219 gram per kilo) injected subcutaneously in the back; 3.40 p. m., convulsion with recovery; 3.50 p. m., frequent spasms with paralysis, especially of anterior extremities; 5.30 p. m., tetanus when removed from cage and put on floor.

October 4: 8.50 a. m., found dead. *Autopsy:* Congestion of small intestines, lungs, liver.

Guinea pig 57. White and gray female. Weight, 350 grams. Diet, oats.

October 3: 2.15 p. m., 3.5 cc 2 per cent caffein injected subcutaneously in the back (0.2 gram per kilo); 3.40 p. m., convulsions with recovery; 5.30 p. m., no marked symptoms.

October 4: 8.50 a. m., alive, active.

October 6: Found dead at 9 a. m. *Autopsy:* Congestion of lungs and liver; kidneys petechiated; severe gastro-enteritis.

Guinea pig 68. Yellow male. Weight, 785 grams. Diet, oats.

October 6: 11.35 a. m., 7.8 cc 2 per cent caffein (0.2 gram per kilo) injected subcutaneously; 12 noon, reflexes increased markedly; 4.20 p. m., reflexes the same as at 12 noon.

October 7: 9 a. m., dead. *Autopsy:* Lungs congested; liver congested and fatty; spleen congested, kidney showed hemorrhagic spots; gastric mucosa necrotic; small portion of small intestine inflamed.

Guinea pig 69. White male. Weight, 585 grams. Diet, oats.

October 6: 11.40 a. m., 5.8 cc 2 per cent caffein injected subcutaneously; 12 noon, reflexes increased, but not as much as in No. 68; 4.20 p. m., guinea pig hypersensitive, reflexes increased more than at 12 noon.

October 7: 9 a. m., alive.

October 15: 9 a. m., found dead.

Guinea pig 61. Brown and black female. Weight, 330 grams. Diet, oats.

October 3: 4 p. m., 4 cc 2 per cent caffein (240 mg per kilo) injected subcutaneously; 5.30 p. m., reflexes increased; runs, but drags posterior extremities.

October 4: 8.50 a. m., found dead.

Guinea pig 62. White, yellow, and black female. Weight, 335 grams. Diet, oats.

October 3: 4.05 p. m., 4 cc 2 per cent caffein (238 mg per kilo) injected subcutaneously in the back; 5 p. m., convulsions; 5.20 p. m., convulsions, alternating with paralysis of anterior and posterior extremities.

October 4: 8.50 a. m., found dead.

Guinea pig 70. White and brown male. Weight, 545 grams. Diet, oats.

October 7: 3 p. m., 6.5 cc 2 per cent caffein (238 mg per kilo) aqueous solution injected subcutaneously; 3.50 p. m., reflexes increased.

October 9: 9 a. m., found dead.

Guinea pig 71. Brown and white male. Weight, 540 grams. Diet, oats.

October 7: 3 p. m., 6.5 cc 2 per cent caffein solution (0.24 gram per kilo) injected subcutaneously; 3.45 p. m., reflexes increased, tetanus.

October 9: 9 a. m., found dead.

Guinea pig 72. Brown and white male. Weight, 560 grams. Diet, oats.

October 7: 3 p. m., 6.5 cc 2 per cent caffein (0.232 gram per kilo) aqueous solution administered by subcutaneous injection; 3.35 p. m., reflexes increased.

October 10: found dead. *Autopsy:* Nos. 70, 71, 72 showed congestion of organs.

The reaction to caffein in the experiments of this series (C) showed considerable variation. The appearance of symptoms, as well as the final outcome of the experiments, differed markedly in a number of cases, notwithstanding the fact that the conditions were the same; thus the administration of 0.2 gram per kilo to guinea pigs, all of which received the same diet, induced no symptoms in two of the animals (Nos. 66 and 65), while marked symptoms were observed in the other four; in two of these the symptoms appeared in one hour and a quarter after injection, and in two others (Nos. 68 and 69), mild symptoms only appeared in 20 or 25 minutes. The last two were under observation for 4 hours longer, but there was no visible change in their condition. The duration of life in all of these guinea pigs, as indicated in the table, likewise varied. Two (Nos. 60 and 68) died during the night after they received caffein, one survived (No. 65), and three others (Nos. 57, 66, and 69) lived 2½, 5, and 9 days, respectively. Experiments with larger doses likewise showed differences in the behavior of these animals toward caffein, but they were not quite so marked. As shown in the table, symptoms appeared in from 35 minutes to 1.5 hours after injection. The duration of life was less than 1 day in two pigs, about twice as long in two others, and in one case between 2 and 3 days.

A comparison made with results obtained in the preceding series shows a striking difference in the resistance to caffein. As 2 decigrams per kilo proved more rapidly fatal to the guinea pig than the larger doses employed in the later experiments, this difference in the resistance to caffein may be due to several factors. As pointed out in the experiments on rabbits, age might be an important factor influencing the toxicity of caffein. Unfortunately, no accurate data were available on the age of the guinea pigs, but they were all apparently full grown, although they differed in weight considerably. The difference in their ages was in all probability not very great. Moreover, it will be observed that the resistance in series B and C differed in animals of approximately the same weight. This is evident on comparing experiments Nos. 20, 38, and 13 of series B with Nos. 65, 66, and 69 of the next series. Again, further inspection and analysis of these tables show no difference in the toxicity, although there may be considerable difference in the weight, from which it may be concluded that the animals were of about the same age or that this plays no part in the resistance to caffein in the guinea pig.

Diet is another factor which should be taken into consideration in this connection. The recent work of Hunt[39] indicates that this may influence the resistance of animals to some poisons. Our experiments, however, fail to show any difference in the toxicity of the caffein in guinea pigs, whether fed oats, carrots, or both, for different results were obtained on the same diet, and there seemed to be little or no difference in the toxicity of caffein when the diet was different. Other explanations suggest themselves to account for the results obtained. Seasonal changes have been assigned by a number of investigators as a cause of variation in the resistance to drugs. According to Focke,[24] frogs are more susceptible to digitalis in the spring than in the summer, while Moschkowitsch [61] and Edmunds [21] reported the very opposite results. Schmiedeberg's [80] observations on strophantin in frogs were in harmony with those of Edmunds [21] and Moschkowitsch.[61] Similar results were reported with guinea pigs. Harrington's [34] experiments indicate that stimulation of the vagus is less effective from October to January than from February to April, when they are also much more susceptible to operative procedure. Hunt found that the resistance of guinea pigs to aceto nitril is about twice as great in the summer months as it is in January and February.

Race might also be thought of as an important factor in this connection. Since the guinea pigs used at different seasons of the year were of several varieties, there is no reason to suppose, however, that the varieties experimented upon in the summer were more resistant than those used in the winter and spring. It is highly probable, therefore, that the greater resistance to caffein of the guinea pigs of series C than those of series B was due to seasonal variation.

Doses of 0.20 to 0.24 gram caffein per kilo weight, therefore, may be regarded as the minimum fatal dose for the guinea pig, depending upon the season. Since 0.2 gram per kilo proved to be rapidly fatal in series B, this quantity was perhaps not the minimum fatal dose for the guinea pig at the season during which the experiments were made. Additional tests with smaller doses were therefore carried out during February and March. The results are shown in series D.

SERIES D.

Guinea pig 49. Male. Weight, 510 grams. Diet, oats for 1 month previous to experiment.

March 17: 3 p. m., 4 cc 2 per cent caffein (0.16 gram per kilo) were injected subcutaneously; 4.40 p. m., reflexes increased; 5.40 p. m., no symptoms.

March 18: 9 a. m., found dead, died in less than 18 hours. *Autopsy:* Hemorrhage into abdominal cavity; liver and spleen unduly congested; intestines injected; hemorrhagic area at point of injection.

Guinea pig 40. Male. Weight, 630 grams. Diet, oats and carrots one week previous to injection.

February 12: 11 a. m., 5 cc 2 per cent caffein (0.158 gram per kilo) injected subcutaneously into back.

February 13: 1 p. m., still alive.

February 14: 9 a. m., found dead.

Guinea pig 45. Female. Weight, 435 grams. Diet, oats for about one month previous to injection.

March 17: 3 p. m., 3.5 cc of 2 per cent caffein injected subcutaneously in the back (0.160 gram per kilo); 4.35 p. m., no symptoms; 5.40 p m., no symptoms.

Guinea pig 39. Male. Weight, 820 grams. Diet, oats and carrots.

February 12: 11 a. m., 6 cc (0.15 gram per kilo) 2 per cent caffein injected subcutaneously in back.

February 14: 9 a. m., alive; seemed to be in good condition; found dead at 1 p. m.

Guinea pig 41. Weight, 660 grams. Diet, oats and carrots one week previous to injection.

February 12: 11 a. m., 5 cc (0.15 gram per kilo) 2 per cent caffein injected subcutaneously.

February 14: 2 p. m., pig alive; apparently normal.

February 18: Guinea pig still alive and apparently in good condition.

Guinea pig 46. Female. Weight, 470 grams. Diet, oats about one month previous to experiment.

March 17: 3.15 p. m., 4 cc (0.170 gram per kilo) 2 per cent caffein injected into back subcutaneously; 4.35 p. m., reflexes increased, tremors on handling marked; 5.40 p. m., no change, symptoms about as before.

March 18: 2.30 p. m., no symptoms.

The experiments of this series (D) likewise showed a considerable difference in the resistance of the individual guinea pigs. Nos. 41, 45, and 46 survived; the rest of the pigs died within 18 hours to 2 days after the administration of caffein. Since an autopsy was held on one only, it is impossible to assign a cause for the variation in the toxicity of caffein in these guinea pigs, as the diet and the other conditions under which the experiments were conducted were the same. It was found in the experiments on cats and rabbits that the presence of morbid processes tends to increase the toxicity of caffein. The observations of Ophüls[66] are of interest in this connection. He found spontaneous lesions of the kidney and liver in a large proportion of guinea pigs examined. The greater susceptibility to caffein of guinea pigs Nos. 39, 40, 49, is probably due therefore to some pathological change which increased its toxicity. About 0.2 to 0.24 gram per kilo may therefore be regarded as the minimum lethal dose for the normal guinea pig when caffein is introduced subcutaneously, the minimum toxic dose being about 150–160 mg per kilo.

Experiments were also conducted to determine the largest dose which does not produce any visible effects. In a number of tests with from 100 to 120 mg caffein per kilo (series E, see Table 6, p. 51) no manifestation of nervous or muscular disturbance nor any departure from the normal in respiratory activity was observed. Such quantities may be regarded as the largest doses which are surely safe for these animals. It is quite possible, therefore, that the greater variation in the toxicity of caffein observed in these experiments is due to morbid conditions. Moreover, there is some evidence that caffein increases the toxicity of certain poisons, as shown by Hale[33] for acetanilid. Is it not possible that caffein may similarly be affected by poisons circulating within the body? Indeed the recent work of Loeb[23] makes this supposition highly probable. This investigator found that caffein and adrenalin injected together produce myocarditis in the rabbit. It is conceivable that the combined action of caffein and some preexisting poison may cause changes which terminate in the death of the animal. The delayed death of guinea pigs after the administration of caffein observed in this and other series may probably be accounted for in this way.

Experiment 57 lends some support to this view. The condition of the kidneys and the presence of a severe gastro-enteritis are sufficient to account for the death of this case. Again the frequent association of gastro-enteritis and congestion of the organs in caffein intoxication found in different animals makes it highly probable that these lesions were caused by caffein.

INJECTION INTO THE PERITONEAL CAVITY.

The experiments were carried out with different doses. All the guinea pigs in this series were kept on a uniform diet, consisting of oats. Most of them were of average size and there were no wide variations in their weights. The experiments of series A with the smallest doses were conducted in March and April; all the other experiments it will be noticed were made in October.

SERIES A.

Guinea pig 41. Weight, 700 grams. Diet, oats.

April 1: 3.30 p. m., 4.5 cc 2 per cent caffein (130 mg per kilo) injected into peritoneal cavity. 5.35 p. m., symptoms present but no tetanus.

April 2: Found dead about 2 p. m., duration of life about 22 hours. *Autopsy:* Subcutaneous hemorrhage at the point of inoculation; serious exudate on visceral and parietal peritoneum with marked inflammation of peritoneum; portions of intestines showed slight enteritis.

Guinea pig 49. Male. Weight, 370 grams. Diet, oats.

April 1: 3.15 p. m., 2.5 cc 2 per cent caffein (135 mg per kilo) injected into the peritoneal cavity; 5.30 p. m., symptoms present; reflexes increased, but no tetanus. Guinea pig survived.

Guinea pig 47. Female. Weight, 550 grams. Diet, oats since about February 4.

March 17: 3.30 p. m., 3.5 cc 2 per cent caffein (127 mg per kilo) injected into peritoneal cavity; 4.35 p. m., increased irritability present, but not marked; 5.40 p. m., symptoms about the same as before.

March 18: 2.30 p. m., condition good; no symptoms. Survived.

Guinea pig 50. Female. Weight, 290 grams. Diet, oats.

April 1: 3.30 p. m., 2 cc 2 per cent caffein (138 mg per kilo) injected into peritoneal cavity; 5.35 p. m., symptoms present; reflexes much increased, but no tetanus. Survived.

SERIES B.

Guinea pig 51. Yellow female. Weight, 415 grams.

October 1: 9.50 a. m., 3 cc (144 mg per kilo) 2 per cent caffein injected into peritoneal cavity; 4.30 p. m., no symptoms, although under observation all day.

October 3: 2 p. m., alive.

Guinea pig 52. White male. Weight, 450 grams.

October 1: 9.45 a. m., 3.5 cc, 2 per cent caffein (155 mg per kilo), injected into peritoneal cavity; 4.30 p. m., no symptoms developed since injection.

October 3: 2 p. m., alive.

Guinea pig 53. Brown and white male. Weight, 490 grams.

October 1: 9.45 a. m., 4 cc, 2 per cent caffein (163 mg per kilo), injected into peritoneal cavity; 4.30 p. m., no symptoms developed since injection.

October 3: 2 p. m., alive.

October 8: Found dead. *Autopsy:* Congestion of lungs, spleen, liver, kidneys, and small intestines.

SERIES C.

Guinea pig 59. Gray and white. Weight, 375 grams. Diet, oats.

October 3: 2 p. m., 3.75 cc (0.2 gram per kilo) injected into peritoneal cavity; 2.15 p. m., reflexes increased but not markedly; 4 p. m., reflexes still more increased; no other symptoms; 5.30 p. m., no symptoms.

October 4: 8.50 a. m., guinea pig alive and active.

Guinea pig 58. Brown and white. Weight, 380 grams. Diet, oats.

October 3: 2 p. m., 3.8 cc caffein (0.2 gram per kilo), 2 per cent solution, injected into peritoneal cavity; 2.10 p. m., hind legs extended, then tetanus; attack lasted a few seconds, after which pig raised himself on his legs, but reflexes remained much exaggerated; 4 p. m. to 5.30 p. m., no symptoms of caffein intoxication.

October 4: 8.50 a. m., guinea pig alive and active.

Guinea pig 56. Gray and white male. Weight, 440 grams. Diet, oats.

October 1: 11.30 a. m., received 4.6 cc of 2 per cent caffein solution (0.2 gram per kilo) into abdominal cavity; 11.45 a. m., stiffness and rigidity of posterior extremities, reflexes increased; 12.30 p. m., hind legs paralyzed, reflexes increased; 4.35 p. m., no symptoms, guinea pig in good condition.

October 3: Still alive, in good condition.

October 14: Died. *Autopsy:* Anterior lobe of right lung hepatized. Small portion of small intestine edematous. Other organs normal.

Guinea pig 55. White and yellow male. Weight, 690 grams. Diet, oats.

October 1: 11.30 a. m., received 6.5 cc of 2 per cent solution caffein (188 mg per kilo) into peritoneal cavity; 11.40 a. m., stiffness in all extremities, reflexes markedly increased; 12.30 p. m., reflexes increased, anterior and posterior extremities paralyzed; 3 p. m., found dead.

SERIES D.

Guinea pig 67. Gray and yellow male. Weight, 330 grams. Diet, oats.

October 5: 11.25 a. m., 4 cc of 2 per cent caffein injected into peritoneal cavity (240 mg per kilo); 11.30 a. m., tetanus—survived, convulsions off and on. Death at 2.55 p. m. *Autopsy:* Severe gastroenteritis; kidney petechiated; congestion of lungs and liver.

Guinea pig 63. Gray and white male. Weight, 340 grams. Diet, oats.

October 5: 11.20 a. m., 4 cc of 2 per cent caffein (235 mg per kilo) injected into peritoneal cavity.

October 14: Alive and in good condition.

Guinea pig 64. Brown and black female. Weight, 305 grams.

October 5: 11.35 a. m., 3.8 cc 2 per cent solution caffein (250 mg per kilo) injected into peritoneal cavity; 11.40 a. m., tetanus—survived, convulsions off and on, died at 4.15 p. m. *Autopsy:* Findings exactly the same as in No. 67.

Examination of the results of the experiments by intraperitoneal injections showed that 0.2 gram caffein per kilo was toxic in two guinea pigs (Nos. 59 and 58). Severe symptoms were observed within 15 minutes in No. 56 and within one hour in No. 55 after the administration of approximately the same dose of caffein. One of these died within three and one-half hours; the other, No. 56, made a good recovery from the acute effects. This amount of caffein may be regarded, therefore, as the minimum toxic dose for the guinea pig when injected into the peritoneal cavity. This is corroborated by the experiments of series B in which smaller doses failed to show any muscular, nervous, or respiratory symptoms, nor were there any after effects noticed, as all of them survived and were kept under observation for some time. The guinea pigs of series A, however, seem to contradict these results. It will be remarked that appreciably smaller doses induced symptoms in all of them, and one case terminated fatally. The seasonal variation, as already pointed out, is in all probability likewise responsible for the difference in the resistance between the guinea pigs of series A and B. Tests were made also to determine the minimum fatal dose. For this purpose the experiments of series D were performed. The resistance of No. 63 in this series is quite striking. We are unable to explain such a discrepancy in the results obtained under practically uniform conditions. The minimum fatal dose of caffein, when injected into the peritoneal cavity, is therefore about 240 to 250 milligrams per kilo. These amounts, it will be observed, were rapidly fatal, in striking contrast to the results obtained when such doses were injected subcutaneously. This is probably due to a better absorption from the peritoneal cavity than from the subcutaneous tissues.

ADMINISTRATION BY MOUTH.

All the guinea pigs in these experiments were kept on a diet of hay and oats and were of large size. The tests were made with different doses of caffein in order to determine the limits of toxicity when the drug was administered by mouth.

Guinea pig 129. White and black male. Weight, 855 grams. Diet, oats and hay.

June 6: 2.20 p. m., 12 cc of 2 per cent caffein (0.28 gram per kilo) by mouth; 3 p. m., reflexes increased; 5 p. m., reflexes still more increased; no other symptoms.

June 7: 9 a. m., found dead; guinea pig passed 75 cc urine, which was almost colorless. *Autopsy:* Heart and blood vessels injected; lungs congested; small intestines congested; other organs apparently normal.

Guinea pig 130. Black and brown male. Weight, 800 grams. Diet, oats and hay.

June 6: 2.30 p. m., 12 cc of 2 per cent caffein (0.3 gram per kilo) administered by mouth; 3 p. m., reflexes increased; 5 p. m., increase of reflexes greater than at 3 p. m.

June 7: 9 a. m., found dead; only a few cubic centimeters of urine passed since 4 p. m. *Autopsy:* Heart and blood vessels injected; lungs congested; small intestines congested slightly.

Guinea pig 131. White and yellow male. Weight, 860 grams. Diet, oats and hay.

June 6: 2.40 p. m., 12 cc 2 per cent caffein administered by mouth; 3 p. m., reflexes increased; 5 p. m., reflexes still more marked.

June 7: 9 a. m., found dead, pig passed about 5 cc urine since 4 p. m. of previous day. *Autopsy:* Same as in No. 130.

Guinea pig 136. White and black male. Weight, 1,000 grams. Diet, oats and hay.

June 9: 4 p. m., 7.5 cc 2 per cent caffein solution injected subcutaneously into the back; 4.50 p. m., reflexes increased.

June 10: 9.30 a. m., more sensitive than normal guinea pigs, but reflexes not quite so marked as at 5 p. m. previous day, about 15 cc urine passed since caffein was injected, reduction of Fehling's solution considerable, no albumin.

June 13: Alive and in good condition. Appetite good. (NOTE.—Parallel test with urine from two guinea pigs which did not receive caffein failed to show reduction of Fehling's solution.)

Guinea pig 137. White and brown male. Weight, 925 grams. Diet, oats and hay.

June 9: 4 p. m., 7 cc 2 per cent solution caffein injected subcutaneously; 4.50 p. m., reflexes increased.

June 10: Reflexes less marked than at 5 p. m. previous day, but is more sensitive than normal guinea pig, about 10 cc urine passed since injection of caffein, moderate amount of reduction of Fehling's solution.

June 13: Guinea pig alive, appetite good, condition good.

June 16: 9 a. m., found dead.

Guinea pig 135. White and black male. Weight, 955 grams. Diet, hay and oats.

June 9: 3 p. m., 7.5 cc 2 per cent caffein solution given by mouth through stomach tube; 4.50 p. m., reflexes increased.

June 10: Reflexes less than on previous day and less marked than in No. 136, a few cubic centimeters dirty brown urine collected but could not be tested for reduction.

June 13: Condition good, appetite good.

June 16: 9 a. m., found dead.

Guinea pig 134. White and brown male. Weight, 740 grams. Diet, hay and oats.

June 9: 2.55 p. m., 6 cc warm 2 per cent caffein solution given by mouth through stomach tube; 4.50 p. m., reflexes increased.

June 10: 9.30 a. m., reflexes much less than day before, increase slight, a few cubic centimeters of urine passed since injection of caffein, looked brown and dirty, could not be tested for reducing substances.

June 13: Guinea pig alive, appetite good, condition good.

June 14: 9 a. m., found dead.

Guinea pig 128. White and black male. Weight, 1,075 grams. Diet, hay and oats.

June 7: 10 a. m., 11 cc 2 per cent caffein by mouth through stomach tube; 11.10 a. m., no symptoms, no urine passed; 1 p. m., increased reflexes, about 15 cc (estimated) urine passed; 4 p. m., reflexes increased, still more urine passed (about 20 cc); 4.50 p. m., tetanus, frequent attacks, then paralysis and death at 4.58 p. m. *Autopsy:* Lungs congested; blood vessels of heart injected; intestines slightly congested; fatty liver.

Guinea pig 126. White and gray male. Weight, 980 grams. Diet, oats and hay.

June 7: 9.40 a. m., 9.8 cc 2 per cent caffein given by mouth through stomach tube; 10 a. m., no symptoms; 11.10 a. m., no urine passed, reflexes increased; 1 p. m., more sensitive than before; 4 p. m., increase of reflexes more marked, no urine passed; 4.45 p. m., about 15 cc urine collected; 5 p. m., no change.

June 8: 9 a. m., reflexes about the same as 5 p. m. previous day, no urine passed since 4.45 p. m. previous day, considerable reduction of Fehling's solution, much more than urine of guinea pig No. 127; 11.05 a. m., convulsions; 12 noon, still alive and stretched out on abdomen; died at 1 p. m. *Autopsy:* Lungs badly congested; heart and blood vessels injected; blood vessels of kidney and of small intestines injected; liver engorged with blood; a few necrotic spots in stomach.

Guinea pig 127. White, black, and brown male. Weight, 760 grams. Diet, oats and hay.

June 7: 9.50 a. m., 7.6 cc 2 per cent caffein by mouth through stomach tube; 10 a. m., no symptoms; 11.10 a. m., reflexes increased, no urine passed; 1 p. m., very sensitive; 4 p. m., sensitiveness increased, about 20 cc urine passed; 5 p. m., no change.

June 8: 9 a. m., reflexes about the same as 5 p. m. previous day; 9.30 a. m., guinea pig passed 30 cc urine since he received caffein, urine showed a moderate amount of reduction; 12 noon, convulsions; died at 2.30 p. m. *Autopsy:* Lungs congested; blood vessels of heart and of intestines injected; numerous necrotic spots in stomach; other organs apparently normal.

Examination of the protocols shows that the absorption of caffein from the gastrointestinal canal was quite rapid, symptoms having been observed as early as 20 minutes after its introduction. The duration of life, it will be remarked, varied with the size of the dose. When approximately 3 decigrams per kilo were fed, all the animals died in the night. They lived, therefore, less than 18 hours. Two decigrams per kilo were likewise fatal, but the duration of life was longer. To decide whether or not this is the smallest fatal dose, smaller amounts were fed. It seemed at first that about 150 mg per kilo was the smallest toxic dose, and about 200 mg per kilo the minimum fatal dose. Macroscopic examination of the organs, however, threw some doubt on this supposition, for well-marked lesions were noticed in all of the guinea pigs which received 0.2 gram per kilo. It is quite possible, therefore, that the minimum fatal dose may be somewhat higher, as we have reason to believe that, at least in some pathologic conditions, the susceptibility to caffein is increased. The presence of fatty changes in the liver of No. 128 and the rapid death in this case lends especial support to this view. Hence, the minimum fatal dose is probably greater than 0.2 gram per kilo for the normal guinea pig. The doses employed for the tests on guinea pigs Nos. 129, 130, and 131 may be considered therefore the minimum fatal dose for these animals. It will be also remarked that macroscopical examination of the organs of these animals failed to reveal the presence of severe lesions. That the minimum toxic dose is probably much smaller than 0.28 gram per kilo is indicated by the experiments on guinea pigs Nos. 135 and 134, in which 0.15 gram caffein per kilo induced mild symptoms in from two to three hours. Both of these, however, and also No. 137 died four to six days after the drug was fed. As already pointed out, caffein may be a factor in the delayed death of guinea pigs which received moderate doses of it. That this supposition may also be true for guinea pigs Nos. 134, 135, and 137 is indeed made probable by the observation that after moderate amounts of caffein symptoms may persist in the guinea pig for about 24 hours, and also by the fact that the secretion of urine in these animals was very scanty, as shown in the preceding record of the experiments; this means slow elimination of caffein and its products of decomposition. It is conceivable that the presence of toxic amounts of caffein in the body for a considerable length of time would induce changes that ultimately lead to the death of the animal or that morbid processes are set up by the combined action of caffein and some preexisting poison. Since some guinea pigs, however, survived the doses indicated, it is more probable that such changes would be brought about by caffein in the presence of a preexisting poison. The death of these pigs, and also of No. 137 several days later, is difficult to account for on any other theory than the one suggested. Were it not for the fact that controls, that is, animals of the same lot which had not received caffein survived all of the experimental animals, changed conditions of environment or accident might be considered the cause of death in the guinea pigs of the last series.

TABLE 6.—*Subcutaneous injection of guinea pigs.*

SERIES A.

Number of pig.	Weight.	Caffein per kilo.	Appearance of symptoms.	Duration of life.	Diet.	Month.	Remarks.
	Grams.	*Gram.*					
18	500	0.300	2 minutes	2 hours 40 minutes	Carrots	March	Female.
15	548	.290		30 minutes	Oats	do	Do.
14	442	.360	15 minutes	do	do	do	Do.
				SERIES B.			
20	497	0.200	2 hours 20 minutes	3 hours 30 minutes	Oats	April	Female.
38	570	.210	25 minutes	2 hours 15 minutes	Carrots	February	Male.
37	820	.200	1 hour 25 minutes	Less than 18 hours	Carrots and oats.	do	Do.
13	618	.194		25 hours	Oats	March	Female.
36	850	.200	1 hour 30 minutes	18 hours	Carrots and oats.	February	Male.
				SERIES C.			
66	510	0.200	None	5 days	Oats	October	Male.
65	510	.200	do	Survived	do	do	Do.
60	320	.219	1 hour 15 minutes	Within 18 hours	do	do	Female.
57	350	.200	do	About 2½ days	do	do	Do.
68	785	.200	25 minutes	Less than 22 hours	do	do	Male.
69	585	.200	20 minutes	9 days	do	do	Do.
61	330	.240	1 hour 30 minutes	Less than 24 hours	do	do	Female.
62	335	.238	1 hour	do	do	do	Do.
70	545	.238	50 minutes	About 2 days	do	do	Male.
71	540	.240	45 minutes	do	do	do	Do.
72	560	.232	35 minutes	About 3 days	do	do	Do.
				SERIES D.			
49	510	0.160	1 hour 40 minutes	Less than 18 hours	Oats	March	Male.
40	630	.158		Less than 2 days	Oats and carrots.	February	Do.
45	435	.160	None	Survived	Oats	March	Female.
39	820	.150		2 days	Oats and carrots.	February	Male.
41	660	.150		Survived	do	do	
46	470	.170	1 hour 20 minutes	do	Oats (?)	March	Female.
				SERIES E.			
19	556	0.100		Survived	Oats	April	
42	490	.120	None	do	do	February	
43	430	.116	do	do	do	do	
44	535	.112	do	do	do	do	
97	330	.100	do	do	do	November	
98	520	.100	do	About 3 days	Carrots	do	

TABLE 7.—*Injection into peritoneal cavity; guinea pigs.*

SERIES A.

Number of pig.	Weight.	Caffein per kilo.	Appearance of symptoms.	Duration of life.	Diet.	Month.	Remarks.
	Grams.	*Gram.*					
41	700	0.130	2 hours	22 hours	Oats	April	Male.
49	370	.135	2 hours 15 minutes.	Survived	do.	do.	Do.
47	550	.127	1 hour	do.	do.	March	Female.
50	290	.138	2 hours	do.	do.	April	Do.

SERIES B.

Number of pig.	Weight.	Caffein per kilo.	Appearance of symptoms.	Duration of life.	Diet.	Month.	Remarks.
51	415	0.144	None	Survived	Oats	October	Female.
52	450	.155	do.	do.	do.	do.	Male.
53	490	.163	do.	do.	do.	do.	Do.

SERIES C.

Number of pig.	Weight.	Caffein per kilo.	Appearance of symptoms.	Duration of life.	Diet.	Month.	Remarks.
59	375	0.200	15 minutes	Survived	Oats	October	Gray and white.
58	380	.200	10 minutes	do.	do.	do.	
56	440	.200	15 minutes	14 days	do.	do.	Male.
55	690	.188	1 hour	3 hours 30 minutes	do.	do.	Do.

SERIES D.

Number of pig.	Weight.	Caffein per kilo.	Appearance of symptoms.	Duration of life.	Diet.	Month.	Remarks.
67	330	0.240	5 minutes	30 minutes	Oats	October	Male.
63	340	.235		Survived	do.	do.	Do.
64	305	.250	25 minutes	4 hours 40 minutes	do.	do.	Female.

TABLE 8.—*Caffein by mouth; guinea pigs.*

SERIES J.

Number of pig.	Weight.	Caffein per kilo.	Appearance of symptoms.	Duration of life.	Diet.	Month.	Remarks.
	Grams.	*Gram.*					
129	855	0.280	40 minutes	Less than 8 hours	Hay and oats	June	Male.
130	800	.300	30 minutes	Less than 18 hours	do.	do.	Do.
131	860	.280	20 minutes	do.	do.	do.	Do.
135	955	.150	1 hour 50 minutes	6 days	do.	do.	Do.
134	740	.160	3 hours	4 days	do.	do.	Do.
[1] 137	925	.150	50 minutes	6 days	do.	do.	Do.
[1] 136	1,000	.150	do.	Survived	do.	do.	Do.
126	980	.200	20 minutes	27 hours	do.	do.	Do.
127	760	.200	1 hour	28 hours	do.	do.	Do.
128	1.075	.200	3 hours	7 hours	do.	do.	Do.

[1] Subcutaneous injection for comparison.

SUMMARY.

A survey of the results obtained in experiments on guinea pigs shows that the mode of introduction of caffein exerts but little influence on its toxicity. On careful analysis it will be observed that the rate of absorption after the administration of caffein by mouth, subcutaneously, or intraperitoneally is about the same for the time of appearance of symptoms. The persistence of the symptoms of caffein intoxication observed in these experiments for 24 hours after administration points to slow elimination, which may be expected, owing to the fact that the guinea pigs passed but little urine and caffein is not diuretic for these animals. The prolonged presence of caffein in the body probably exerts a harmful influence or after effect, which may account for the delayed death of some animals many days after a single dose of caffein was given. Among the factors which undoubtedly influence toxicity, season should be considered, while the presence of a diseased condition undoubtedly tends to decrease the resistance of the guinea pig to caffein. Diet was without any influence on the toxicity of the single dose of caffein.

EXPERIMENTS ON CATS.

These experiments were performed on well-fed animals which were kept under observation for several days before the tests with caffein were made. The diet consisted of meat exclusively. In some cases the urine was examined for albumin and sugar before caffein was given. No tests with caffein were made if large amounts of albumin were found. It may be remarked that sugar was never found in cats before the administration of caffein, but that considerable amounts of it were found in some cases after it was given. Studies by various modes of administration were made, by subcutaneous injection, intraperitoneally, or by mouth. Attention was also directed to the resistance to caffein in young cats, several experiments on kittens being made with this object in view.

SUBCUTANEOUS INJECTION.

Rost stated that caffein is eliminated in the urine unchanged after its introduction into the body and that the amounts found varied with different species of animals. In the rabbit the amount eliminated was about 21 per cent; in the dog about 8 per cent; and in the cat somewhat less than 2.5 per cent. It would appear, therefore, that the cat decomposes caffein more readily than the rabbit or dog; its resistance consequently ought to be greater than that of the other animals. Moderately large doses were accordingly employed in the preliminary experiments (series A), but the results obtained, as shown in the protocols, indicated that caffein is fully as toxic for the cat as for the rabbit or dog. The doses were then decreased and experiments were performed in order to ascertain the smallest toxic as well as the smallest fatal dose.

Series A

Three decigrams of caffein per kilo were administered in these experiments. The results are shown in the following protocols:

Cat 4. Black and white. Weight, 1,440 grams.

May 26: 10.05 a. m., 22 cc 2 per cent caffein (0.3 gram per kilo) injected subcutaneously; 11.10 a. m., copious salivation, cat irritable, muscular stiffness present, but no tetanus; 11.45 a. m., cat restless, convulsions, attacks of short duration, no paralysis observed after the convulsions, pupils dilated; 4.45 p. m., cat quiet, slight paralysis present.

May 27: Cat exhausted.

May 28: Found dead.

Cat 5. Black and white male. Weight, 1,396 grams.

June 3: 10 a. m., 21 cc of 2 per cent caffein (0.3 gram per kilo) injected subcutaneously; 12 noon, found dead.

Although there was considerable difference in the duration of life following the injection of the same dose of caffein per kilo, the final outcome was the same, as both cats died from the effects of the drug. One died within 2 hours and the other lived more than 30 hours after its administration. Three decigrams of caffein per kilo is, therefore, surely fatal to these animals. Tests made with smaller doses are shown in the following experiments:

Series B.

In these experiments the doses employed ranged between 0.20 and 0.25 gram caffein per kilo.

Cat 3. Black and white female. Weight, 2,854 grams. Well fed.

June 4: 10.30 a. m., 35 cc 2 per cent caffein (0.25 gram per kilo) injected subcutaneously; 11 a. m., found dead.

Cat 6. Black and white. Weight, 1,645 grams.

June 3: 20 cc 2 per cent caffein (0.243 gram per kilo) injected subcutaneously at 3 p. m., cat grew very irritable in a few minutes; about 4. p. m. reflexes decidedly increased; 5 p. m., cat paralyzed.

June 4: Cat found dead.

Cat 8. Weight, 1,735 grams.

October 7: 4 p. m., 22 cc 2 per cent caffein (0.25 gram per kilo) injected subcutaneously in the back; 4.30 p. m., cat irritable, salivation profuse, convulsions; died at 5.30 p. m.; no urine passed after caffein was given.

Cat 9. Weight, 1,960 grams.

October 7: 3.45 p. m., 25 cc 2 per cent caffein (0.25 gram per kilo) injected subcutaneously in the back; 4.45 p. m., cat very irritable, repeated attacks of convulsions, salivation copious; died at 5.30 p. m.; cat did not urinate after injection of caffein.

Cat 12. Striped kitten. Weight, 1,185 grams.

October 9: Urine examined, no albumin, no sugar; 1.45 p. m., 12 cc 2 per cent caffein administered; 5 p. m., cat alive, no symptoms except salivation and general irritability.

October 10: 10.30 a. m., found dead. About 15 cc urine collected, but no examination made.

Cat 14. Black. Weight, 1,855 grams.

October 8: 1.40 p. m., 18.5 cc 2 per cent caffein (0.2 gram per kilo); 3 p. m., cat became restless about 10 minutes after caffein was injected; cried persistently and moved about in cage, no convulsions, cat urinated about 15 cc, cat defecated.

October 9: 9 a. m., cat found dead in cage. Urine gave very heavy reduction of Fehling's solution (much more than was obtained from urine of rabbits); 20 cc urine analyzed contained 4.65 per cent sugar. *Autopsy:* Lungs deeply congested; liver marked fatty infiltration and degeneration; spleen normal; kidneys pale and anemic; intestines normal; stomach normal.

Cat 15. Striped. Weight, 2,145 grams.

October 8: 2 p. m., 22 cc (0.2 gram per kilo) 2 per cent caffein injected subcutaneously; 2.30 p. m., cat irritable, restless, trying to get out of cage, crying persistently; 2.40, convulsions lasting about two minutes, then cat raised itself and made attempts to get out of cage, no salivation, cat urinated about 10 cc and defecated.

October 9: 9 a. m., cat found dead in cage, about 10 cc of urine contained enormous quantities of sugar. *Autopsy:* Lungs severely congested; liver showed marked fatty degeneration; spleen normal; kidneys slightly pale and anemic; intestines mildly congested; stomach normal.

Cat 19. White. Weight, 1,100 grams.

October 20: 13 cc of 2 per cent caffein (0.236 gram per kilo). About 15 minutes later cat became irritable, reflexes increased, persistent crying, stiffness of extremities, diarrhea present; 4.30 p. m., stiffness of muscles, coordination much disturbed, walked with great difficulty; 4.30 p. m., no new symptoms, persistent crying continued.

October 21: Found dead.

Cat 20. White kitten. Weight, 790 grams.

October 20: 11.35 a. m., 10 cc 2 per cent caffein (0.25 gram per kilo) given subcutaneously; 12 noon, convulsions followed by paralysis; 1.30 p. m., still breathing, apparently in comatose condition, lay on its side, dyspnoea, profuse salivation; 4 p. m., convulsions and death.

The results of the experiments of series B show that a dose of even 0.2 caffein per kilo is very toxic for the cat. Symptoms appeared in one animal 40 minutes after the injection of caffein. Some of them were found dead 18 hours after injection, which means that the duration of life was probably a great deal less since there was evidence that they had been dead for some time. Death occurred quite soon after larger doses were injected. Cat No. 3 died 30 minutes after it received caffein. The amounts employed in these experiments can not be considered therefore as the minimum fatal doses. Smaller doses were then tried, as shown in the experiments of the next series.

SERIES C.

Experiments were performed on five cats which received from 140 to 155 mg per kilo, as follows:

Cat 24. Striped. Weight, 1,300 grams.

October 25: 10 a. m., 50 cc urine, albumin moderate amount—no sugar; 10 cc caffein injected subcutaneously at 12 noon; 12.30, irritable, cried persistently, no appetite; 4 p. m., no convulsions, but persistent crying.

October 27: Cat was still alive.

Cat 17. Weight, 2,620 grams.

October 12: 9.30 a. m., 65 cc urine collected; more than a trace of albumin present, no reduction of Fehling's solution; 3 p. m., 20 cc 2 per cent caffein (150 mg per kilo) injected subcutaneously; 3.15 p. m., irritable and restless.

October 13: 9 a. m., about 15 cc urine collected, reduction of Fehling's solution marked; osazone test also positive.

Cat 23. Black and white. Weight, 1,645 grams.

October 25: 10 a. m., 140 cc urine collected (since October 23), small amount of albumin present, no sugar.

October 27: 9 a. m., no albumin; no sugar in urine; 11.50 a. m., 12.5 cc caffein injected subcutaneously (0.15 gram per kilo); 1 p. m., convulsions and death.

Cat 7. Striped kitten. Weight, 1,285 grams.

October 11: Urine collected, no albumin, no sugar; 9.50 a. m., 10 cc 2 per cent caffein injected subcutaneously in the back; 10.10, violent convulsions lasting about 30 seconds; 10.20, convulsions of shorter duration; 10.30 convulsions; 10.35, convulsions lasting a few seconds; urine passed about 10.20, contained a moderate amount of albumin, but there was no reduction of Fehling's solution; 10.45, profuse salivation and paralysis; died about 10.50.

Cat 39. Yellow. Weight, 2,285 grams.

April 13: 2.40 p. m., 16 cc 2 per cent caffein (0.14 gram per kilo) injected subcutaneously in the back; 3.45 p. m., cat died.

Of the five experiments of this series three died after doses of 140, 150, and 155 mg per kilo. The other two showed symptoms of toxicity, but survived a dose of 150 mg per kilo which indicated that the minimum fatal dose was probably reached. To test this supposition smaller doses were administered, as shown in the following experiments.

SERIES D.

Ten cats were used for this series of experiments, and the doses administered varied between 103 and 139 mg per kilo. The results shown in the appended table (p. —) indicate that about 120 to 140 mg of caffein per kilo may induce mild symptoms in some cases. The conclusion may be safely drawn therefore that 150 mg per kilo is approximately the minimum fatal dose for the cat when the drug is given subcutaneously. That smaller doses are, however, by no means to be regarded as always safe is shown in the following experiments.

SERIES E.

Cat 43. Weight, 3,225 grams.[a]

September 14: 10.20 a. m., 20 cc 2 per cent caffein (0.124 gram per kilo) injected into the back; 11 a. m., tetanus and death. *Autopsy:* Lungs congested; liver congested and showed hemorrhagic spots in capsules and fatty degeneration; kidneys slightly congested; other organs normal.

Cat 48. Black female. Weight, 3,050 grams.

September 14: 18 cc 2 per cent caffein (0.118 gram per kilo) injected subcutaneously in the back; 10.30 a. m., violent convulsions and death. *Autopsy:* Lungs congested in spots showing numerous petechia; liver congested; spleen congested; other organs normal.

The diminished resistance to caffein of cats Nos. 43 and 48 might be due to the pathologic changes found on autopsy, for evidence is not wanting that the toxicity of drugs might be greatly altered under pathological conditions. Hunt[40] has shown that resistance to acetonitril is considerably diminished in chronic alcoholism. This seems to be true also of other drugs under abnormal conditions. Smaller doses of atropin[78] are required in lead poisoning than under normal conditions to produce the same results. The following experiment is of interest in this connection, for in this case a much smaller dose than was given in experiments Nos. 43 and 48 produced the typical symptoms of caffein poisoning and proved to be fatal.

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[a] Cat probably old; had been in the laboratory for several weeks before the experiment. Gained in weight 175 grams.

Cat 47, black and white male. Weight, 4,220 grams.
September 15: Received subcutaneously 18 cc 2 per cent caffein (0.084 gram per kilo); no symptoms observed for about six hours.
September 16: No symptoms.
September 17: Weight, 4,250 grams; injected 18 cc 2 per cent caffein (0.084 gram per kilo); tetanus and death after two hours. *Autopsy:* Severe hemorrhagic pneumonia; kidneys pale, other organs normal.

Since two controls survived the same dose in proportion to the body weight of the animal without showing any symptoms, the assumption is justified that the lower resistance to caffein was due to the presence of pneumonia, thus affording additional support to the view that the toxicity of caffein may be increased in disease.

INJECTION INTO THE PERITONEAL CAVITY.

These experiments were carried out on full-grown and on young subjects. As in previous experiments, doses of different sizes were employed. A dose of 0.2 gram per kilo was tried first and then reduced gradually to 0.1 gram per kilo.

Cat 10. Female. Weight, 2,970 grams.
October 9, 1909: 1.30 p. m., 30 cc 2 per cent caffein (0.2 gram per kilo) injected into the peritoneal cavity; urine examined for albumin and sugar, negative; cat found dead at 2.30 p. m. No urine in the bladder.

Cat 16. Black female. Weight, 2,420 grams.
October 9, 1910: Urine examined for albumin and sugar, negative; 2.30 p. m., 22 cc 2 per cent caffein (0.183 gram per kilo) injected into the peritoneal cavity; found dead at 3 p. m.

Cat 99. Well-fed gray female. Weight, 3 kilos.
June 22, 1911: 3.40 p. m., 15 cc 2 per cent caffein injected into peritoneal cavity; salivation and marked irritability within one hour after injection.
June 24: Alive, appetite good.

Cat 98. Well-fed black male. Weight, 4,100 grams.
June 22: 3.45 p. m., 20.5 cc 2 per cent caffein (0.1 gram per kilo) injected into peritoneal cavity; very irritable a few minutes after injection, no other symptoms.
June 24: No symptoms, appetite good.

Cat 93. Black and white. Weight, 1,450 grams.
June 22: 3 p. m., 30 cc 2 per cent caffein (0.137 gram per kilo) injected into peritoneal cavity; salivation, no other symptoms; under observation until 6 p. m.
June 23: 9 a. m., no urine, cat showed no symptoms.
June 24: 9 a. m., no symptoms, took nourishment as usual.

Cat 87. Well-fed white female. Weight, 2,615 grams.
June 23: 2.45 p. m., 19 cc 2 per cent caffein (0.145 gram per kilo) injected into peritoneal cavity; became irritable and restless.
June 24: 9 a. m., no symptoms, took nourishment as usual.

Cat 97. Gray. Age, 3 months. Weight, 500 grams. Diet, meat.
June 24: 2.25 p. m., 5 cc 2 per cent caffein (0.2 gram per kilo) injected into peritoneal cavity; 4 p. m., no symptoms.
June 29: Died.

Cat 96. Gray and white. Age, 3 months. Weight, 575 grams. Diet, meat.
June 24: 2.20 p. m., 4 cc 2 per cent caffein (0.139 gram per kilo) injected into peritoneal cavity; 3.55 p. m., no symptoms.
June 30: Died.

Cat 95. Black. Age, about 3 months. Weight, 860 grams. Diet, meat.
June 24: 10.15 a. m., 8.6 cc 2 per cent caffein injected into peritoneal cavity, salivation immediately after injection; 10.25, convulsions and paralysis; died 10.45 a. m. *Autopsy:* Macroscopical examination of the organs, negative.

Cat 94. Black and white. Weight, 790 grams. Age, about 3 months. Diet, meat.
June 24: 10 a. m., 8 cc 2 per cent caffein injected into peritoneal cavity; 4 p. m., under continual observation since injection, cat very irritable, respiration more rapid than normal, diarrhea present.

Examination of the above protocols show that a dose of 2 decigrams per kilo was fatal within one hour to one cat and that a somewhat smaller dose killed another individual in 30 minutes. Amounts under 0.15 gram per kilo were just sufficient to induce mild symptoms, such as increased irritability and salivation, which disappeared within a few hours. In no case were the effects noticeable on the following day. The experiments on young kittens are especially interesting, as they proved, contrary to expectation, to be distinctly more resistant than full grown individuals. The death of Nos. 97 and 96 within five and six days, respectively, can not be ascribed to caffein, since some of the controls also died. Moreover, it will be remarked in this connection that no symptoms appeared in three of the four young kittens after the administration of a dose which was rapidly fatal to adult cats. The rapid death of No. 95 after the same dose forms an exception which can not be accounted for, as macroscopical examination at autopsy proved negative.

ADMINISTRATION BY MOUTH.

Two decigrams per kilo were given at first, but it was found that this amount was surely fatal. The dose was therefore reduced to 0.125 gram per kilo. In all of these experiments caffein was given by means of a soft rubber catheter slipped over the stem of a funnel which served as a stomach tube. A 2 per cent aqueous solution was used throughout these tests except in one case in which caffein was given mixed with the food.

Cat 92. Black and white female. Weight, 1,750 grams.
June 10: 12.05 p. m., 14 cc 2 per cent caffein (0.16 gram per kilo) given by mouth; cat was quiet when tied on holder, struggled only a little when tube was put into stomach; 12.30 p. m., cat vomited, no other symptoms.
June 13: Condition good, appetite good.

Cat 87. White female. Weight, 2,620 grams. Diet, meat.
June 5: 2.15 p. m., 20 cc 2 per cent caffein (0.15 gram per kilo) solution administered by mouth through stomach tube; 2.30 p. m., cat irritable, but no other symptoms; 5 p. m., condition about the same, except that it was more irritable and showed some stiffness of the extremities.
June 13: Alive and in good condition, appetite good, not irritable.

Cat 91. White female. Weight, 3,050 grams.
June 10: 12 noon, 23 cc (0.15 gram per kilo) of 2 per cent caffein administered by mouth, cat struggled violently; 1.30, salivation; 1.40 p. m., convulsions; died at 2 p. m. *Autopsy:* Congestion of lungs, liver, and spleen; heart vessels injected; other organs normal.

Cat 88. Black and white female. Weight, 3,260 grams. Diet, meat.
June 5: 2.20 p. m., 25 cc of 2 per cent caffein (0.15 gram per kilo) given by mouth; 2.45 p. m., cat irritable, no other symptoms (cat did not vomit after the administration of caffein); 4 p. m., cat found dead. *Autopsy:* Liver very much congested; heart contracted; body was still warm at the time of autopsy.

Cat 90. White and yellow female. Weight, 2,685 grams. Diet, meat.
June 5: 3.15 p. m., 27 cc of 2 per cent caffein (0.2 gram per kilo) given by mouth through stomach tube, about half an hour later cat became irritable and began to salivate; at 4.30 p. m. salivation became more marked, dyspnœa was well developed, and the cat was quite restless and had tremors; 5 p. m., short spasms of posterior extremities, but lay quietly in the cage most of the time; 5.20 p. m., convulsions of short duration and death, muscular relaxation followed immediately after convulsions, no vomiting, diarrhea observed after administration of caffein, and cat passed about 10 cc of urine.
June 6: 9 a. m., found dead.

Cat 89. White and black female. Weight, 2,860 grams. Diet, meat.
June 5: 3.15 p. m., 28.6 cc (0.2 gram per kilo) of 2 per cent caffein given by mouth through stomach tube, no vomiting observed, nor any other symptoms; 3.30, found dead. *Autopsy:* Organs normal; liver congested.

Cat 82. Gray female. March 3, weight 2,450 grams; June 6, weight 2,750 grams. Diet, 150 grams of meat daily.

June 7: Given 0.4125 gram of caffein in 150 grams of meat, did not eat.

June 8: Given 0.4125 gram of caffein in 150 grams of meat, refused to eat.

June 9: Given 150 grams of meat without caffein, ate all of it.

June 10: No food given.

June 11: No food given.

June 12: Given 0.4125 gram caffein in 150 grams of meat (150 mg per kilo), ate all of it.

June 13: Found dead. *Autopsy:* Lungs congested, liver congested; other organs apparently normal.

Cat 100. Gray female. Weight, 2,740 grams. Diet, meat.

July 17: 3 p. m., 17 cc of 2 per cent caffein (124 mg per kilo) given by mouth through stomach tube at 3.20 p. m.; 5 p. m., very irritable, but no other symptoms.

July 18: Under observation all day, no symptoms.

Cat 93. Black and white female. Weight, 1,640 grams.

July 17: 3.30 p. m., 10 cc (0.125 gram per kilo) of 2 per cent caffein given by mouth through stomach tube.

July 18: Under observation all day, no symptoms.

From the results of the experiments of this series it appears that 0.15 gram caffein per kilo may be fatal within a few hours after its administration, even if the drug is mixed with a moderate amount of meat. Experiments 87 and 92 show, however, that this amount may be borne by some individuals without any serious consequences, as the cats were under observation for some time after they received caffein, and no untoward symptoms were noticed in either of them during this time. It may be remarked that cat No. 92 vomited shortly after it received caffein. It is practically certain, therefore, that this amount of caffein in proportion to the weight of the animal will in the great majority of cases prove fatal, and perhaps in a smaller percentage of individuals it is surely toxic if it does not escape absorption. Smaller doses may cause irritability in some individuals, but symptoms referable to nervous symptoms of muscles were absent, as in experiments Nos. 93 and 100. The minimum fatal dose of caffein for the cat when given by mouth is, therefore, 0.15 gram per kilo.

TABLE 9.—*Subcutaneous injection; cats.*

Number.	Weight.	Caffein per kilo.	Symptoms.	Duration of life.
			SERIES A.	
	Grams.	*Gram.*		
4	1,440	0.30	65 minutes	Over 29 hours.
5	1,396	.30		About 2 hours.
			SERIES B.	
3	2,854	0.25		30 minutes.
6	1,645	.243	Few minutes	1 hour 30 minutes.
8	1,735	.25	30 minutes	1 hour.
9	1,960	.25	1 hour	1 hour 45 minutes.
12	1,185	.20	3 hours	Less than 18 hours.
14	1,855	.20	1 hour 20 minutes	Do.
15	2,145	.20	40 minutes	Do.
19	1,100	.236	15 minutes	Do.
20	790	.25		4 hours 30 minutes.
			SERIES C.	
24	1,300	0.153	1 hour	Survived.
17	2,620	.15	15 minutes	Do.
23	1,645	.15		1 hour 10 minutes.
7	1,285	.155	20 minutes	1 hour.
9	2,285	.14		65 minutes.

TABLE 9.—*Subcutaneous injection; cats*—Continued.

SERIES D.

Number.	Weight.	Caffein per kilo.	Symptoms.	Duration of life.
	Grams.	*Gram.*		
13	730	0.139	Restlessness	
21	1,165	.138	None	
25	965	.103	do.	
26	1,605	.125	do.	
27	1,625	.125	do.	
28	2,335	.128	do.	Received 2 doses; survived.
40	2,710	.129	do.	Do.
41	1,785	.123	do.	Do.
42	2,315	.112	do.	Do.
38	2,325	.120	Mild	Died after second dose.

SERIES E.[1]

43	3,225	0.124		40 minutes.
48	3,050	.118		Died soon after.
47	4,220	.084		Survived.
[2] 47	4,250	.084		2 hours.

[1] Pathological conditions.

[2] Two days after first injection.

TABLE 10.—*Injections into peritoneal cavity; cats.*

Number.	Weight.	Caffein per kilo.	Symptoms.	Duration of life.
	Grams.	*Gram.*		
99	3,000	0.100	Mild	Survived.
98	4,100	.100	do.[1]	Do.
93	1,450	.137	Very mild	Do.
87	2,615	.145	do.	Do.
97	505	.200	None	5 days.
96	575	.139	do.	6 days.
95	860	.200	15 minutes	30 minutes.
94	790	.200	Diarrhea	Survived.
10	2,970	.200		1 hour.
16	2,420	.183		30 minutes.

[1] In few minutes.

TABLE 11.—*Administration of caffein by mouth; cats.*

No.	Weight.	Caffein per kilo.	Symptoms.	Duration of life.
	Grams.	*Gram.*		
91	3,050	0.15	1 hour 40 minutes	2 hours.
88	3,260	.15		1 hour 40 minutes.
92	1,750	.16	25 minutes	Survived.
87	2,620	.15	3 hours	Do.
90	2,685	.20	1 hour 15 minutes	Less than 18 hours.
89	2,860	.20		75 minutes.
82	2,450	.15		Less than 24 hours.
100	2,740	.124	1 hour 40 minutes	Survived.
98	1,640	.125		Do.

SUMMARY.

The toxicity of caffein in cats is shown to be the same when given by mouth as when injected subcutaneously, the minimum fatal doses in both cases being 0.15 gram per kilo. When introduced by the intraperitoneal route, caffein is, on the contrary, distinctly less toxic. After the administration of 0.137 and 0.145 gram caffein per kilo (Nos. 93 and 87) salivation in one cat (No. 93) and irritability and muscular stiffness in the other were the only effects noticed. These symptoms were no longer observed the next day and the cats appeared to be perfectly normal. Experiments with larger doses indicate that the minimum fatal dose by this method of administration is about 0.2 gram per kilo.

EXPERIMENTS ON DOGS.

The experiments were carried out on well-fed adult dogs and on puppies, kept under observation for some time before the drug was administered. Only those manifesting no signs of abnormality were used for these tests. Caffein was given by mouth mixed with 10 to 20 grams of meat, or subcutaneously in 2 per cent aqueous solution. The young animals received caffein dissolved in milk. The determination of the minimum toxic or fatal doses when the drug was fed presented considerable difficulty, as in many instances the ingestion of the drug was closely followed by vomiting.

ADMINISTRATION BY MOUTH.

SERIES A.

The effective dose in these experiments showed considerable variation. One dog (No. 38) died after a dose of 0.12 gram caffein per kilo, while some subjects survived doses of 0.2 and 0.23 gram per kilo. In the 12 experiments given in Table 12, page 62, it will be noticed that from 0.12 to 0.152 gram per kilo proved fatal to three dogs, while three others survived the same amounts in proportion to the body weight. The results were the same with larger doses. It may be observed in this connection that in the case of the five dogs in which vomiting was noticed some time during the 24 hours following the administration of caffein, four survived, No. 38 being the exception. The greater toxicity of caffein in this case is in all probability due to some morbid process, the presence of which was indicated by the high temperature of this subject.

That vomiting may avert a fatal issue after larger doses of caffein is made further probable by experiment on dog No. 48, for which, in the absence of vomiting, a dose of 0.2 gram of caffein per kilo proved fatal. On this supposition the discrepancy in the results obtained in this series may be readily explained. The smallest doses which proved fatal in these experiments were 0.145 and 0.152 gram per kilo. No. 38, which died from a dose of 0.12 gram per kilo, may be considered as an exception, as this subject was not normal. Experiments with caffein on dogs were made at various other times in this laboratory but failed to show that smaller doses of caffein, even when vomiting did not occur after its administration, were fatal, although toxic effects were observed. The conclusion is therefore justified that the minimum fatal dose of caffein for the normal dog is about 0.15 gram per kilo when given by mouth.

SUBCUTANEOUS INJECTION.

SERIES B.

To determine the toxicity of caffein more accurately, especially for comparison with animals of other species, the subcutaneous method of administration was also used. The injections were made with a syringe of 20 cc capacity, the contents of which were introduced into contiguous areas. The results of experiments on six dogs indicate that approximately 150 to 160 mg per kilo is the minimum fatal dose, since such doses proved fatal to two out of the three animals receiving this amount, while three others which received doses of from 143 to 160 mg per kilo survived.

EXPERIMENTS ON PUPPIES.

SERIES C.

In these experiments the resistance of young growing puppies to caffein was studied. Caffein was given by mouth to all the subjects except one, to which it was administered subcutaneously. The protocols, only a few of which are given, and the tabulated data of the experiments (p. 62) show that the age of the animal has a decided influence on the toxicity of caffein.

Dog 11. Weight, 1,260 grams.

August 2: At 10 a. m. given 12.5 cc of 2 per cent of caffein through stomach tube; 2 p. m., had convulsions, diarrhea, salivation, and stiffness of limbs.

August 3: Found dead 9 a. m. *Autopsy:* Thoracic viscera apparently normal; stomach immensely distended and filled with a white, cheesy mass and some fluid; round worms plentiful in stomach and small intestine; mucosa of entire intestine congested; contents of lower intestine congested; liver pale; spleen flabby; kidney congested.

Dog 10. Weight, 1,650 grams.

July 26: 9.30 a. m., 29 cc of 2 per cent caffein added to 60 cc of milk offered, but refused, and was therefore fed by mouth through stomach tube; 10.25 a. m., no symptoms; 11.30 a. m., restlessness, extremities stiff, post. extremities spread apart, dog shows well-marked symptoms of caffein poisoning; 12.10 p. m., symptoms more severe, extremities extended and spread out, is lying flat on belly so that nose touches floor of the cage; 12.40 p. m., found dead; was alive at 12.10 p. m. *Autopsy:* Lungs showed hemorrhagic foci in all lobes; heart apparently normal; liver fatty; stomach and intestines filled with round worms; spleen and kidney apparently normal.

Dog 9. Weight, 3,000 grams.

July 25: 350 mg caffein per kilo; 5 p. m., lying down most of the time, occasionally walks about in stall; restlessness present, but not marked; 5.30 p. m., vomit which looked frothy and mucilaginous noticed on the floor of the stall; no meat particles noticed in vomit, though searched for; whines occasionally.

July 26: 9 a. m., looks well; no signs of the effect of caffein given the day previous.

Dog 8. Yellow female. Weight, 3,100 grams.

July 22: 10.50 a. m., received 1.1 grams of caffein in 10 grams of meat (354 mg caffein per kilo); 3 p. m., vomited mucus; gait clumsy; refused to eat; continually drinking water; very restless; 4 p. m., convulsions set in at 3.55 p. m.; tonic rigidity of the posterior extremities; profuse salivation; convulsions were both tonic and clonic in character, and resembled those seen in rabbits in caffein intoxication; a striking feature was the duration of the spasm, which began at 3.55 p. m. and kept up for more than two hours.

July 23: Found dead at 9 a. m.

The data recorded in the table and in the protocols of the experiments of series C show that four out of the seven animals experimented upon died in less than 24 hours after caffein was fed; three of these received 300 to 354 mg caffein per kilo, and one received 200 mg caffein per kilo. No. 8 vomited four hours after caffein was given. No vomiting was observed in the other three dogs. From 0.300 to 0.350 gram of caffein per kilo may be regarded, therefore, as surely fatal to young growing puppies. That this is in all probability the minimum lethal dose appears from the following experiments: No. 9, which received 350 mg per kilo, vomited one hour after and survived, which indicates that some of it was probably not absorbed. The amount which entered the circulation was therefore less than 350 mg per kilo. Since No. 15, which received 250 mg caffein subcutaneously, likewise survived, the probabilities are that 300 to 350 mg per kilo were the minimum fatal doses for these animals. Moreover, No. 12, which received 200 mg caffein per kilo, survived, no vomiting having been observed. The case of No. 11, in which the same amount of caffein in proportion to body weight proved fatal, may be explained perhaps by the findings of the autopsy.

The results obtained in these experiments justify the conclusion that young growing dogs can stand larger doses of caffein than full-grown and older dogs.

Attention may also be called here to the difference in the symptoms produced by caffein in very young and in adult dogs. It was often noticed in these experiments that the symptoms in older subjects when given toxic doses of caffein set in rather abruptly and ended in instantaneous death. We failed to observe this phenomenon after the administration of large amounts of caffein to very young dogs, in which tonic and clonic convulsions alternating with paresis were observed. These symptoms set in rather gradually and lasted several hours (see experiment No. 8), resembling the rabbit in this regard.

SUMMARY.

The toxicity of caffein for adult dogs is about the same, whether given by mouth or injected subcutaneously. The resistance of puppies to caffein is much greater than that of adults.

TABLE 12.—*Administration by mouth; dogs.* (*Series A.*)

No.	Weight.	Caffein per kilo.	Results.	Remarks.
	Kilos.	*Gram.*		
47	13.60	0.144	Survived	Vomiting observed.
55	12.75	.200	do	Stiffness of muscles; no other symptoms.
56	7.95	.200	Found dead next day	
52	13.60	.147	Survived	
57	6.50	.230	do	Vomited after 1 hour; convulsions after 1 hour and 45 minutes.
39	23.10	.120	do	Increased frequency of respiration, thirst, loss of appetite, vomited rest of day when he drank water, salivation, restlessness, passed feces frequently.
48	11.50	.174	do	Vomiting observed.
48	12.00	.200	Found dead next day	No vomiting observed. Second dose was given 8 days after first.
54	13.40	.200	Survived	Vomiting observed.
49	13.15	.152	Found dead next day	
38	14.50	.120	do	Symptoms after 1½ hours: Dog had a temperature of 104° F. before caffein was given; vomited 3 hours after caffein was fed.
18	10.30	.145	do	

TABLE 13.—*Subcutaneous injection; dogs.* (*Series B.*)

No.	Weight.	Caffein per kilo.	Results.	Remarks.
	Kilos.	*Gram.*		
62	9.30	0.161	Survived	Restlessness and vomiting one-half hour after injection.
61 A	14.00	.160	Found dead next day	
63	12.00	.150	Survived	Restlessness 1 hour after injection.
64	14.00	.150	do	Restlessness and thirst 45 minutes after injection.
59	7.20	.160	Died 1 hour and 20 minutes after injection.	Marked restlessness, but no convulsion.
61	14.60	.143	Survived	Symptoms observed in 1½ hours.

TABLE 14.—*Administration by mouth to puppies.* (*Series C.*)

No.	Weight.	Caffein per kilo.	Result.	Remarks.
	Kilos.	*Gram.*		
8	3.10	0.354	Found dead next day	Vomited in 4 hours after feeding; restlessness, loss of appetite, thirst, incoordination of muscles, convulsions.
9	3.15	.350	Survived	Muscular incoordination and stiffness, restlessness, vomited 1 hour after caffein was given.
10	1.60	.350	Died in 3 hours	Convulsion; no vomiting.
11	1.26	.200	Found dead next day	Salivation; convulsions.
12	1.28	.200	Survived	No symptoms.
15	1.20	.250	do	Subcutaneous injection.
16	3.50	.300	Died in 1 hour	Convulsions 45 minutes after caffein was fed.

CHRONIC CAFFEIN INTOXICATION.

The object of this study was to ascertain the effect of repeated dosage when caffein was given daily or at longer intervals. The experiments were tried on rabbits and on dogs. As in the experiments on acute intoxication, the animals were under observation for some time in the laboratory before the administration of caffein was begun in order to ascertain the presence or absence of abnormality. The relation of diet to toxicity received some attention, but the question was not studied exhaustively in the present investigation.

EXPERIMENTS ON RABBITS.

Full-grown adult as well as young rabbits were employed. The diet consisted either of carrots or of oats; water was given ad libitum. The rabbits were kept in metal cages in a well-lighted and well-ventilated room. Unnecessary handling or any other procedure tending to fatigue or to cause discomfort to the animals was very carefully avoided, since we had found that such treatment was likely to decrease the resistance of the rabbit to caffein. The caffein was administered by feeding by mouth and through a stomach tube, or by the subcutaneous method. In a good many cases it was given daily, in some at longer intervals.

SERIES A.

The experiments of this series formed a preliminary study for the purpose of testing the effect of moderate doses. One decigram of caffein per kilo was given daily for several days; when administered at longer intervals the dose was increased to 150 to 200 mg per kilo. It was found that the smaller doses did not produce any symptoms; even the weights of the animals were not influenced. Doses of medium size given on two successive days were likewise without any noticeable effect (Nos. 182, 183, 123, 101). When a third dose of this size was given within 48 or 24 hours it proved fatal (Nos. 123, 182, and 183). Exceptionally, however, moderately large doses (for rabbits) may be given for three consecutive days without fatal issue, as in rabbit No. 101. When given at intervals of two to three days, larger doses, as may be seen from the protocols, can be administered without causing acute death (Nos. 173, 181, 201).

The results of the tests of this series point to the absence of any accumulation and to the possible elimination of moderate doses of caffein and its products of decomposition within 24 hours or thereabouts. When the doses are larger the time of its elimination is apparently longer, as shown by the fact that repetitions of the dose the next day may be fatal, but when a longer interval is allowed it may be given without causing death. It will be observed that only one rabbit of this series survived, but it was extremely emaciated. This condition has been observed in a number of cases after caffein had been given for several days. Even when the drug was withdrawn the animals continued to lose weight. This may be explained by the condition of the gastro-intestinal canal as found at autopsy. The presence of inflammation of the mucous membrane of the stomach and intestines, with ulceration of the mucous membrane of the pylorus in one of the rabbits (No. 173) of the series, in all probability caused diminution or loss of appetite, which of itself would tend to cause loss of flesh and strength and finally death. Protocols of the experiments follow.

Rabbit 173. Carrots were fed from October 1 to 18 and oats for the remainder of the experiment.

Date.	Weight.	Caffein per kilo.	Date.	Weight.	Caffein per kilo.
	Grams.	*Gram.*		*Grams.*	*Gram.*
Oct. 9	1,980	0.141	Oct. 16	2,005	0.220
Oct. 11	1,905	.190	Oct. 18	1,845	....
Oct. 13	1,930	.207	Oct. 20	1,740	.230

October 21: Paralysis of posterior extremities.

October 22: 9 a. m., found dead.

The urine was examined before and after the administration of caffein. No symptoms were observed after the administration of caffein (5 doses in 11 days), nor was albumen or sugar found in the urine after any of the experiments on this rabbit. *Autopsy:* Pyloric mucosa exhibited several ulcers; small intestines showed slight inflammation; liver deeply congested; kidneys showed marked inflammation of cortex; other organs practically normal.

Rabbit 181. Diet, carrots September 29–October 17, then oats.

Date.	Weight.	Caffein per kilo.	Date.	Weight.	Caffein per kilo.
	Grams.	*Gram.*		*Grams.*	*Gram.*
Oct. 4	1,425	0.105	Oct. 11	1,370	0.175
Oct. 5	1,450	.100	Oct. 13	1,385	.180
Oct. 6	1,270	.100	Oct. 16	1,345	.200
Oct. 7	1,210	.100	Oct. 17	1,030	....
Oct. 8	1,375	.130	Oct. 18	1,230	....
Oct. 9	1,305	.153	Oct. 20	1,215	....

Rabbit was markedly emaciated and weak. No albumen or sugar found in the urine as a result of caffein feeding.

Rabbit 182. Diet of carrots from September 29.

Received caffein subcutaneously as follows:

Date.	Weight.	Caffein per kilo.	Date.	Weight.	Caffein per kilo.
	Grams.	*Gram.*		*Grams.*	*Gram.*
Oct. 4	1,765	0.100	Oct. 8	1,685	.135
Oct. 5	1,880	.100	Oct. 9	....	.150
Oct. 6	1,750	.100	Oct. 11	1,605	.174
Oct. 7	1,710	.100			

October 12: 11 a. m., 23 hours after caffein was given, convulsions with recovery; rabbit died at 1.30 p. m. No sugar was found in the urine at any time after the administration of caffein. Albumen was present only in one specimen.

Rabbit 183. Diet of carrots from September 29.

Received caffein subcutaneously as follows:

Date.	Weight.	Caffein per kilo.	Date.	Weight.	Caffein per kilo.
	Grams.	*Gram.*		*Grams.*	*Gram.*
Oct. 4	1,385	0.100	Oct. 8	1,310	0.153
Oct. 5	1,460	.100	Oct. 9	1,390	.142
Oct. 6	1,385	.100	Oct. 11	1,390	.187
Oct. 7	1,240	.122			

October 12: 9 a. m., found dead. No albumen was found in the urine. Only one sample contained sugar.

Rabbit 123. White, female. Diet, oats.
Received caffein subcutaneously as follows:

Date.	Weight.	Caffein per kilo.	Date.	Weight.	Caffein per kilo.
	Grams.	*Grams.*		*Grams.*	*Grams.*
Apr. 14	2,350	42	Apr. 20	2,126	141
Apr. 16	2,250	90	Apr. 21	1,965	152
Apr. 17	2,325	86	Apr. 22	1,876	160

Rabbit died 30 minutes after last injection of caffein. *Autopsy:* Stomach exhibited marked inflammation of mucosa. Slight enteritis. Liver and kidneys were deeply congested and dark colored.

Rabbit No. 101, white male. Diet, oats.
Received caffein subcutaneously as follows:

Date.	Weight.	Caffein per kilo.	Date.	Weight.	Caffein per kilo.
	Grams.	*Gram.*		*Grams.*	*Gram.*
Mar. 18	2,025	0.100	Mar. 24	1,815	0.166
Mar. 19	1,970	.100	Mar. 25	1,830	.185
Mar. 20	2,009	.100	Mar. 26	1,710	.176
Mar. 22	1,855	.100	Mar. 29	1,734	.219
Mar. 23	1,738	.114	Apr. 1	1,606	.224

April 5: Found dead. *Autopsy*: Marked inflammation of gastric mucosa. Considerable enteritis affecting the whole extent of the intestines; liver congested and friable; kidneys deeply congested in cortical and medullary portions; spleen congested, but of normal size; lungs and heart normal.

Four days, 0.1 per kilo; 10 doses in 14 days.

Rabbit 201. Diet of carrots begun October 1; October 19, oats.
Subcutaneous injections as follows:

Date.	Weight.	Caffein per kilo.	Date.	Weight.	Caffein per kilo.
	Grams.	*Gram.*		*Grams.*	*Gram.*
Oct. 9	1,000	0.150	Oct. 16	1,065	0.225
Oct. 11	1,015	.180	Oct. 18	850	....
Oct. 13	1,065	.187	Oct. 20	890	.111

Under observation six hours October 20; no symptoms.

October 23: Died; was much emaciated but did not show any symptoms; emaciation set in when caffein was withdrawn; urine never contained sugar or albumen; symptoms observed after second dose only.

SERIES B.

The question whether caffein is cumulative in the rabbit, suggested in the preceding experiments, was the subject of further investigation in Series B. Caffein was given by mouth or subcutaneously. Carrots formed the exclusive diet, a measured amount being given. The rabbits were kept under observation for two weeks, except Nos. 370 and 373, records of which were made only for four days before the administration of caffein was begun. Caffein was given by mouth in experiments of Groups I and III. Rabbits 292, 293, and 295 received daily 20 cc water by mouth for four days previous to the administration of caffein, while in the rabbits of Group II the caffein treatment was preceded by the injection of 0.8 per cent salt solution subcutaneously. The object in both cases was to ascertain whether or not the method of the administration of caffein has any influence on the animal, but observation made from day to day failed to show any effect of such treatment. About 1 decigram of caffein per kilo was administered daily, with occasional intermissions. Later in the course of the experiment the doses were increased, 0.15 gram per kilo being the maximum dose given. Rabbit 293 died after the third dose with symptoms of typical caffein poisoning. The administration of the same dose of caffein was continued 10 days longer in Nos. 292 and 295. It was omitted on the seventh, fourteenth, and fifteenth days of the experiment. On the eighteenth day of the experiment the dose was increased to 150 mg per kilo and was repeated 2 days later. No. 295 was found dead the next day. No. 292 survived. Rabbits 313 and 315 may be considered together, as they were treated alike in every respect. The initial dose of 100 mg caffein per kilo was finally increased to 122 mg. After the twelfth dose the emaciation was well marked and the rabbits were very weak. No. 313 was found dead 2 days, and No. 315 3 days, after the last dose of caffein was given. It should be remarked in this connection that symptoms of caffein poisoning were never observed in these rabbits. Death was not due, therefore, primarily to caffein, but the rapid loss of flesh and strength observed during the last few days suggests that it was due to malnutrition apparently brought about by caffein.

The results obtained by subcutaneous injection of caffein are given in the table as Group II. The initial dose of 100 mg per kilo was injected daily. No. 298 died after the second dose. Nos. 223 and 296 received this amount daily for 6 days. An intermission of 2 days followed, at the end of which the same dose was given again. The next day it was increased to 150 mg per kilo, but no effect was observed; 48 hours later this dose was repeated. No. 223 was found dead, but its mate survived. Symptoms of acute caffein intoxication were not observed in any of these rabbits. It would seem, therefore, that caffein is not cumulative. This supposition, however, appears somewhat contradictory in view of the fact that out of the eight rabbits of this series six died, nor could any cause of death be ascribed other than caffein. Also the first results of Experiments 293, 370, and 373 might be considered as indicating that cumulation, though to a moderate extent, does take place, since in these cases reflexes developed after the drug was given for some time. But this view is contradicted by the results of Experiment 371, in which 150 mg per kilo given 5 days after the daily dosage of caffein was suspended likewise caused increased reflexes. Cumulation, therefore, does not account for the effects noted in the other rabbit. It will be observed that rabbit No. 370, as well as Nos. 371 and 373, had diarrhea for several days. It is quite possible that the weakened condition rendered the rabbits more sensitive to the action of the drug. This is made highly probable by the observations recorded in the experiments on acute intoxication with caffein in which death occurred after small doses. In such cases some pathological condition was often disclosed by the autopsy. The results of this series corroborate, therefore, those of Series A, and indicate again the absence of cumulative action. The results obtained are in all probability due to malnutrition and other conditions brought about by congestion of the viscera and consequent injury to the gastro-intestinal canal.

TABLE 16.—*Chronic caffein intoxication of rabbits; Series B on cumulation.*

Data.	Group I.			Group II.			Group III.	
	No. 292.	No. 293.	No. 295.	No. 296.	No. 223.	No. 298.	No. 315.	No. 313.
Diet (grams carrots in 2 days)	1,000	1,000	975	930	905	880	355	300
Caffein administered (cc) and weight (grams):								
Mar. 5	 1,410	 1,470	 1,045	 1,040	 1,070	 955	 770	 770
Mar. 7	 1,415	 1,360	 1,140	 1,090	 1,095	 1,000	 715	 690
Mar. 9	 1,350	 1,270	 1,070	 1,000	 1,055	 1,005	 655	 665
Mar. 11	 1,505	 1,465	 1,190	 1,230	 1,285	 1,250	 755	 760
Mar. 16	 1,580	 1,460	 1,230	 1,165	 1,170	 1,145	 730	 745
Mar. 17	 1,515	 1,415	 1,080	 1,040	 1,115	 1,105	 720	 685
Mar. 19	 1,565	 1,570	 1,280	 1,195	 1,235	 1,220	 710	 735
Mar. 21	7 1,585	7 1,530	6 1,265	 1,150	 1,215	 1,260	4 755	4 700
Mar. 22	7 1,440	7 1,315	6 1,175	(1) 1,100	(1) 1,045	(1) 1,150	4 675	4 635
Mar. 23	7 1,335	7 1,140	6 1,110	(1) 1,145	(1) 1,190	(1) 1,230	4 715	4 700
Mar. 24	7 1,310	.... (2)	6 1,090	(1) 1,115	(1) 1,170	(1) 1,250	4 680	4 650
Mar. 25	7 1,375	....	6 1,035	(1) 1,125	(1) 1,215	(1) 1,215	4 695	4 685
Mar. 26	7 1,255	....	6 1,095	 1,105	 1,155	 1,150	4 675	 695
Mar. 27	....	....	....	....	....	....	....	....
Mar. 28	7 1,355	....	6 1,115	5.5 1,120	6 1,160	6 1,155	4 595	4 685
Mar. 29	7 1,385	....	6 1,150	6 1,155	6 1,165	5 955	4 695	4 675
Mar. 30	7 1,330	....	6 1,075	6 1,035	6 1,095	.... Dead.	4 630	4 610
Mar. 31	7 1,325	....	6 1,170	6 1,110	6 1,140	....	4 690	4 605
Apr. 1	7 1,335	....	6 1,050	6 1,050	6 1,120	....	4 625	4 620
Apr. 2	7 1,390	....	6 1,125	6 1,090	6 1,155	....	4 695	4 625
Apr. 3	....	....	....	....	....	....	 200	....
Apr. 4	 1,300	....	 1,005	 1,105	 1,080	....	 585	 580
Apr. 5	7 1,385	....	6 1,090	6 1,130	6 1,090	....	4 655	4 630
Apr. 6	9 1,260	....	7.5 1,010	7.5 1,050	8 1,110	....	 560	 530
Apr. 7	....	....	....	....	....	....	....	.... (3)
Apr. 8	9 1,260	....	7.5 1,000	7.5 1,090	8 1,065	....	.... Dead.	....
Apr. 9	Survived.	....	Dead.	Survived.	Dead.	....	....	Dead.

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[1] On these days 5 cc of salt solution was administered subcutaneously.

[2] Dead Mar. 23.

[3] Found dead 9 a. m.

TABLE 17.—*Chronic intoxication of rabbits, series B, Group IV, on cumulation.*

RABBIT, 370.

Date.	Weight.	Carrots.	Water.	Urine.	Caffein by stomach.	Symptoms.
	Grams.	*Grams.*	*cc.*		*Mg per kilo.*	
Aug. 7	2,155	450	50	280		
Aug. 8	2,030	450	25	185		
Aug. 9	2,105	290	0	275		
Aug. 10	2,095	450	30	335		
Aug. 11	2,105	450	65	360	50	
Aug. 12	2,125	450	65	220	50	
Aug. 13	2,120	350	25	265	50	
Aug. 14	2,170	450	35	275	75	
Aug. 15	2,175	350	(?)	200	75	
Aug. 16	2,170	360	65	250	75	
Aug. 17	2,175	310	35	170	100	
Aug. 18	2,095	180	40	285	100	Severe diarrhea.
Aug. 19	2,120	400	(?)	285	125	Do.
Aug. 20	2,120	400	(?)	310	125	Better.
Aug. 21	2,120	400	70	250	125	Do.
Aug. 22	2,040	400	45	265	150	Diarrhea bad.
Aug. 23	2,030	370	35	220	150	Diarrhea better.
Aug. 24	1,950	215	40	120	150	Do.
Aug. 25	1,885	195	35	60	200	Reflexes.
Aug. 26						Found dead at 9.

RABBIT, 373.

Aug. 7	2,240	450	50	230		
Aug. 8	2,150	150	30	300		
Aug. 9	2,120	205	0	150		
Aug. 10	2,150	450	15	245		
Aug. 11	2,195	450	5	285	50	
Aug. 12	2,160	450	65	325	50	
Aug. 13	2,120	300	45	190	50	
Aug. 14	2,195	450	40	265	75	
Aug. 15	2,215	350	35	200	75	
Aug. 16	2,205	310	45	225	75	
Aug. 17	2,240	400	40	265	100	
Aug. 18	2,255	350	30	320	100	
Aug. 19	2,115	185	(?)	170	125	Severe diarrhea.
Aug. 20	2,115	280	35	195	125	Diarrhea better.
Aug. 21	2,050	175	75	115	125	Slight diarrhea.
Aug. 22	2,060	180	75	130	150	
Aug. 23	2,005	200	75	125	150	Reflexes.
Aug. 24	1,990	200	75	150	150	Slight diarrhea.
Aug. 25	1,950	255	55	132	175	Severe diarrhea.
Aug. 26	1,870	205	80	140	None	Do.
Aug. 27	1,830	200	50	140	do	Do.
Aug. 28	1,950	400	25	265	do	Slight diarrhea.
Aug. 29	1,825	400	0	315	do	Very weak and in poor condition.
Aug. 30	1,850		10	140	do	
Aug. 31	1,835					

TABLE 17.—*Chronic intoxication of rabbits, series B, Group IV, on cumulation*—Contd.

RABBIT, 371.

Date.	Weight.	Carrots.	Water.	Urine.	Caffein.	Symptoms.
	Grams.	*Grams.*	*cc.*		*Mg per kilo.*	
Aug. 7	2,240	450	50	300	....	
Aug. 8	2,260	450	50	225	....	
Aug. 9	2,310	430	(?)	300	....	
Aug. 10	2,295	450	50	305	....	
Aug. 11	2,320	450	50	335	50	
Aug. 12	2,280	450	70	400	50	
Aug. 13	2,300	350	70	255	50	
Aug. 14	2,265	425	55	154	75	
Aug. 15	2,260	250	40	125	75	
Aug. 16	2,295	155	70	Lost....	75	
Aug. 17	2,180	105	70	120	100	Severe diarrhea.
Aug. 18	2,150	125	70	100	100	Diarrhea better.
Aug. 19	2,075	210	(?)	192	100	Diarrhea severe.
Aug. 20	2,075	280	70	180	100	Do.
Aug. 21	2,165	260	50	225	None....	Diarrhea better.
Aug. 22	2,105	400	50	275	...do.....	
Aug. 23	2,080	300	0	145	...do.....	Diarrhea severe.
Aug. 24	2,105	250	15	245	...do.....	Do.
Aug. 25	2,055	320	10	176	150	Reflexes.
Aug. 26	2,040	190	75	250	150	Died at 1 p. m., without having showed any symptoms other than reflexes.

SERIES C.

The subjects used in these experiments were rabbits of medium size and were apparently young or at any rate were not very old. The series was planned for the study of the possible effect of diet on the toxicity of caffein when given for some time, and therefore oats were substituted for carrots, which had been fed in the previous work, as already stated. Caffein was given by mouth in the usual way, in 1 per cent solution, 100 mg. per kilo daily. Fourteen rabbits were used for these tests. Their weights were recorded daily and observations made at frequent intervals during the day.

The only change noticed in all of the experiments of this series was progressive loss of weight which set in from 3 to 8 days after the administration of the drug was begun. The duration of life varied considerably. No. 382 died after the first dose. No. 389 lived 2 days, No. 386, 3 days, and No. 385, 5 days, No. 390 lived 7 days and No. 404 lived 20 days after the administration of caffein was begun. The duration of life in all the others was from 11 to 16 days. The findings at autopsy are interesting and suggestive as regards the possible explanation of the effects of repeated dosage of caffein. In eight of the rabbits there was involvement of the mucous membrane of the stomach or intestines or of both. Since the same condition of the gastro-intestinal canal was observed in previous experiments with caffein when injected subcutaneously, the mere passing of the tube into the stomach is obviously not the cause of this condition. The fatal outcome due is therefore, as was suggested above, to inanition brought about by the condition of the gastro-intestinal canal. Moreover parallel experiments carried out on rabbits in the same way with alcohol survived this treatment much longer. Obviously then the passing of the soft rubber catheter is not the cause of this condition of the gastro-intestinal canal nor the diet. Rabbits were fed oats exclusively for several months in this laboratory and thrived. The presence of pneumonia in the other rabbits of this series may be regarded as accidental, as it is inconceivable that one or two doses of caffein, as was the case in Nos. 382 and 389, could predispose the lungs to infection. The results of these experiments therefore are in harmony with those of the preceding two series, indicating that caffein does not accumulate in the body, and that the toxicity of caffein, whether of the single dose or of repeated doses is the same, on a diet of carrots or of oats. These results also show that caffein is much more toxic with repeated dosage. As stated in the historical part of this bulletin the same view was held by Gourewitch.[28]

Rabbit 386. Belgian female.
Given 1 cc of 1 per cent caffein for each 100 grams, through stomach tube.

Date.	Weight.	Treatment.	Date.	Weight.	Treatment.
	Grams.	*cc.*		*Grams.*	*cc.*
Aug. 17	1,300	13.0	Aug. 19	(?)	(?)
Aug. 18	1,215	12.0			

August 20: Found dead 9 a. m. *Autopsy*: Lungs slightly congested; liver engorged and friable; gall cyst well filled.

Rabbit 389. Black male.
Given 1 cc of 1 per cent caffein for each 100 grams, through stomach tube.

Date.	Weight.	Treatment.	Date.	Weight.	Treatment.
	Grams.	*cc.*		*Grams.*	*cc.*
Aug. 17	1,070	10.0	Aug. 18	1,025	10.0

August 19: Found dead 9 a. m. *Autopsy*: Lungs severely congested and partially hepatized; liver was engorged; other organs appeared normal.

Rabbit 382. Belgian female.
On August 17 weighed 1,035 grams; received 1 cc of 1 per cent caffein for each 100 grams; 10 cc of 1 per cent caffein given in all.

August 18: Found dead 9 a. m. *Autopsy*: Lungs congested and hepatized; liver engorged; stomach showed numerous petechial hemorrhages on mucosa; kidneys slightly congested; intestines appeared normal.

Rabbit 385. Belgian female.
Given 1 cc of 1 per cent caffein for each 100 grams, through stomach tube.

Date.	Weight.	Treatment.	Date.	Weight.	Treatment.
	Grams.	*cc.*		*Grams.*	*cc.*
Aug. 17	780	8.0	Aug. 20	715	7.0
Aug. 18	760	7.5	Aug. 21	700	7.0
Aug. 19	755	7.5			

August 22: Found dead 9 a. m. *Autopsy*: Lungs exhibited pneumonic lesions, with inflammation of adjacent pleura; a fibro-plastic exudate present around lung; liver showed a coccidial infestation; stomach distended with ingesta; mucous membrane characterized by a catarrhal inflammation; contents of small intestine liquid in nature and bile stained; large intestine somewhat impacted; liver and kidneys seemingly normal.

Rabbit 404. White male.

Given 1 cc 1 per cent caffein for each 100 grams.

Date.	Weight.	Treatment.	Date.	Weight.	Treatment.
	Grams.	*cc.*		*Grams.*	*cc.*
Aug. 20	1,465	14.5	Aug. 30	1,320	13.0
Aug. 21	1,475	14.5	Aug. 31	1,330	13.5
Aug. 22 [1]			Sept. 1	1,335	13.5
Aug. 23	1,475	14.5	Sept. 2	1,315	13.0
Aug. 24	1,400	14.0	Sept. 3	1,350	13.5
Aug. 25 [2]	1,405	14.0	Sept. 4	1,335	13.5
Aug. 26	1,415	14.0	Sept. 5	1,350	13.5
Aug. 27	1,400	14.0	Sept. 6	1,380	14.0
Aug. 28 [1]			Sept. 7	1,375	14.0
Aug. 29	1,310	13.0	Sept. 8	1,325	13.0

[1] Not fed. [2] Reflexes.

September 9: Found dead 9 a. m. *Autopsy*: Both lungs showed extensive pneumonia, with adhesions to pleura; pleuritis and pericarditis very marked; large amount of fibrous exudate in pleural cavity; pyloric end of stomach slightly congested; liver congested; other organs normal.

Rabbit 393. Belgian.

Given 1 cc of 1 per cent caffein to each 100 grams, through stomach tube.

Date.	Weight.	Treatment.	Date.	Weight.	Treatment.
	Grams.	*cc.*		*Grams.*	*cc.*
Aug. 17	950	9.5	Aug. 25	835	8.5
Aug. 18	910	9.0	Aug. 26	780	8.0
Aug. 19	895	9.0	Aug. 27	765	7.5
Aug. 20	910	9.0	Aug. 28 [1]		
Aug. 21	905	9.0	Aug. 29	710	
Aug. 22 [1]			Aug. 30 [2]		
Aug. 23	825	8.0	Aug. 31 [1]		
Aug. 24	870	8.5			

[1] Not fed. [2] Condition very poor; not fed.

September 1: Found dead. *Autopsy*: Lungs congested and adhering to the pleura; extensive inflammation of pleura; liver slightly enlarged and congested; mucosa of stomach and small intestines slightly congested; other organs normal.

Rabbit 390. Belgian, male.

Given 1 cc of 1 per cent caffein to each 100 grams through stomach tube.

Date.	Weight.	Treatment.	Date.	Weight.	Treatment.
	Grams.	*cc.*		*Grams.*	*cc.*
Aug. 17	1,490	15.0	Aug. 21	1,265	12.5
Aug. 18	1,370	14.0	Aug. 22 [1]		
Aug. 19	1,365	13.5	Aug. 23	1,120	11.0
Aug. 20	1,340	13.5			

[1] Not fed.

August 24: Found dead 9 a. m. *Autopsy:* Heart and lungs appeared normal; abdominal viscera showed no apparent pathologic change other than coccidial infection of the liver and fullness of the blood vessels.

Rabbit 392. Maltese, female.
Given 1 cc of 1 per cent caffein to each 100 grams through stomach tube.

Date.	Weight.	Treatment.	Date.	Weight.	Treatment.
	Grams.	*cc.*		*Grams.*	*cc.*
Aug. 17	1,265	12.5	Aug. 26	1,140	11.5
Aug. 18	1,275	12.5	Aug. 27	1,140	11.5
Aug. 19	1,240	12.5	Aug. 28 [1]	....	....
Aug. 20	1,220	12.0	Aug. 29	1,115	11.0
Aug. 21	1,245	12.5	Aug. 30	1,080	11.0
Aug. 22 [1]	....	....	Aug. 31	1,020	10.0
Aug. 23	1,180	12.0	Sept. 1	995	10.0
Aug. 24	1,190	12.0	Sept. 2	930	9.0
Aug. 25	1,155	11.5			

[1] Not fed.

Died at 3 p. m. September 2. *Autopsy:* The stomach and small intestines showed numerous small hemorrhagic spots; a thick coating of mucus surrounded the contents of the stomach; the other organs were apparently normal.

Rabbit 403. Black.
Given 1 cc of 1 per cent caffein for each 100 grams.

Date.	Weight.	Treatment.	Date.	Weight.	Treatment.
	Grams.	*cc.*		*Grams.*	*cc.*
Aug. 20	1,640	16.5	Aug, 26	1,390	14.0
Aug. 21	1,640	16.5	Aug. 27	1,330	13.5
Aug. 22 [1]	....	....	Aug. 28 [1]	....	....
Aug. 23	1,490	15.0	Aug. 29	1,130	11.5
Aug. 24	1,515	15.0	Aug. 30	1,055	10.5
Aug. 25	1,475	15.0			

[1] Not fed.

August 31: Found dead at 3 p. m. *Autopsy:* Extensive gastroenteritis; liver enlarged and congested; spleen slightly congested; peritoneum thickened and congested; other organs normal.

Rabbit 384. Black, female.
Given 1 cc of 1 per cent caffein for each 100 grams through stomach tube.

Date.	Weight.	Treatment.	Date.	Weight.	Treatment.
	Grams.	*cc.*		*Grams.*	*cc.*
Aug. 16	1,195	12.0	Aug. 25	990	10.0
Aug. 17	1,205	12.0	Aug. 26	960	9.5
Aug. 18	1,140	11.5	Aug. 27	955	9.5
Aug. 19	1,180	12.0	Aug. 28 [1]	....	....
Aug. 20	1,145	11.5	Aug. 29	870	9.0
Aug. 21	1,145	11.5	Aug. 30 [2]	850	8.5
Aug. 22 [1]	....	....	Aug. 31	810	8.0
Aug. 23	1,005	10.0	Sept. 1	740	7.5
Aug. 24	1,035	10.5			

[1] Not fed.
[2] Poor condition, mucus from rectum.

September 2: Found dead at 9 a. m. *Autopsy:* The mucosa of stomach showed numerous hemorrhagic spots; the first portion of the small intestines was slightly congested; the other organs were apparently normal in appearance.

Rabbit 383. Belgian, female.

Given 1 cc of 1 per cent caffein for each 100 grams through stomach tube.

Date.	Weight.	Treatment.	Date.	Weight.	Treatment.
	Grams.	*cc.*		*Grams.*	*cc.*
Aug. 16	995	10.0	Aug. 22[1]	....	....
Aug. 17	1,005	10.0	Aug. 23	875	9.0
Aug. 18	990	10.0	Aug. 24	855	8.5
Aug. 19	895	9.0	Aug. 25	850	8.5
Aug. 20	945	9.5	Aug. 26	785	8.0
Aug. 21	965	9.5	Aug. 27	710	7.0

[1] Not fed.

August 28: Found dead at 9 a. m. *Autopsy:* Lungs, heart, and spleen apparently normal; liver infected with coccidia; stomach apparently normal; walls of small intestines injected; colon marked congestion and hemorrhagic; kidneys hemorrhagic.

Rabbit 387. Belgian male.

Given 1 cc of 1 per cent caffein for each 100 grams through stomach tube.

Date.	Weight.	Treatment.	Date.	Weight.	Treatment.
	Grams.	*cc.*		*Grams.*	*cc.*
Aug. 17	1,260	12.5	Aug. 26	1,185	12.0
Aug. 18	1,340	13.0	Aug. 27	1,255	12.5
Aug. 19	1,335	13.0	Aug. 28[1]	....	....
Aug. 20	1,300	13.0	Aug. 29	1,115	11.0
Aug. 21	1,325	13.0	Aug. 30	1,135	11.5
Aug. 22[1]	....	....	Aug. 31	1,175	12.0
Aug. 23	1,205	12.0	Sept. 1	1,050	10.5
Aug. 24	1,200	12.0	Sept. 2	900	9.0
Aug. 25	1,285	12.5			

[1] Not fed.

September 3, found dead. *Autopsy:* Stomach and small intestines showed numerous hemorrhagic spots; thick coating of mucus surrounded the contents of the stomach; bladder was greatly distended with urine; the other organs were apparently normal.

Rabbit 388. Belgian male.

Given 1 cc of 1 per cent caffein for each 100 grams, through stomach tube.

Date.	Weight.	Treatment.	Date.	Weight.	Treatment.
	Grams.	*cc.*		*Grams.*	*cc.*
Aug. 17	1,080	10.0	Aug. 23	1,020	10.0
Aug. 18	1,115	11.0	Aug. 24	985	10.0
Aug. 19	1,150	11.5	Aug. 25	960	9.5
Aug. 20	1,130	11.5	Aug. 26	900	9.0
Aug. 21	1,120	11.0	Aug. 27	875	9.0
Aug. 22[1]	....	....	Aug. 28[1]	....	....

[1] Not fed.

August 29, found dead 9 a. m. *Autopsy:* Heart and lungs normal; liver and kidneys engorged; stomach normal; intestines showed a catarrhal inflammation, though not severe; spleen normal; walls of colon somewhat injected.

Rabbit 391. Belgian.
Given 1 cc of 1 per cent caffein to each 100 grams through stomach tube.

Date.	Weight.	Treatment.	Date.	Weight.	Treatment.
	Grams.	*cc.*		*Grams.*	*cc.*
Aug. 17	940	9.5	Aug. 24	805	8.0
Aug. 18	950	9.5	Aug. 25	800	8.0
Aug. 19	955	9.5	Aug. 26	765	7.5
Aug. 20	935	9.5	Aug. 27[2]	690	7.0
Aug. 21	945	9.5	Aug. 28[1]	....	....
Aug. 22[1]	....	....	Aug. 29	565	5.5
Aug. 23	835	8.5			

[1] Not fed. [2] Poor condition.

August 30, found dead 9 a. m. *Autopsy:* Heart injected; lungs normal; liver affected slightly with coccidiidea; stomach normal in appearance; small intestines normal, but colon considerably inflamed; kidneys slightly engorged; other organs normal.

Rabbit 402. Black female.
Given 1 cc of 1 per cent caffein to each 100 grams.

Date.	Weight.	Treatment.	Date.	Weight.	Treatment.
	Grams.	*cc.*		*Grams.*	*cc.*
Aug. 20	2,030	20.0	Aug. 27	1,765	17.5
Aug. 21	1,950	19.5	Aug. 28[1]	....	....
Aug. 22[1]	....	....	Aug. 29	1,630	16.5
Aug. 23	1,955	19.5	Aug. 30	1,540	15.5
Aug. 24	1,905	19.0	Aug. 31	1,510	15.0
Aug. 25	1,890	19.0	Sept. 1	1,425	14.0
Aug. 26	1,780	18.0			

[1] Not fed.

September 2, found dead 9 a. m. *Autopsy:* The lungs were badly congested, the posterior lobe of the right lung showing hepatization; the liver was considerably enlarged and congested; the mucous membrane of the stomach and small intestines was congested and showed numerous hemorrhagic spots; the kidneys showed slight congestion; all other organs normal.

SERIES D.

The evidence brought forth in the preceding pages regarding cumulation of caffein naturally suggests the question whether or not the body acquires a tolerance for it. This question has already been answered in the affirmative by Gourewitch,[28] but owing to the method he used for the identification of caffein and the few experiments made his results are not conclusive. The experiments of series A, B, and C might be regarded as indicating that tolerance for caffein is not acquired by the rabbit. It was noticed, however, that the rabbit apparently does tolerate increasingly larger doses under certain conditions, as the following experiments show:

Rabbit 223. Belgian hare, male.

October 22: Weight, 1,520 grams; 15 cc 2 per cent caffein injected subcutaneously at 2 p. m.

November 1: 10.30 a. m., weight, 1,510 grams; 17 cc 2 per cent caffein injected subcutaneously (225 mg per kilo), reflexes observed, but no tetanus.

November 4: 10.30 a. m., weight 1,535 grams; 19 cc 2 per cent caffein injected subcutaneously at 2.40 p. m.; 4.40 p. m., no symptoms.

November 8: Weight, 1,425 grams; 20 cc 2 per cent caffein (285 mg per kilo) injected at 11.45 p. m.; 5 p. m., no symptoms.

November 17: Weight, 1,325 grams; 22 cc 2 per cent caffein injected at 2.55 p. m. (329 mg per kilo), no symptoms.

November 18: Rabbit in good condition.

Rabbit 224. Belgian hare, female. Diet, carrots.

October 18: Weight, 1,935 grams; 11.20 a. m., 15 cc 2 per cent caffein (155 mg per kilo) injected.

November 1: Weight, 1,780 grams; 20 cc 2 per cent caffein (224 mg per kilo) injected subcutaneously, reflexes increased, muscle tremors present, but no other symptoms.

November 4: Weight, 1,710 grams; 21.5 cc 2 per cent caffein (252 mg per kilo) injected.

November 8: Weight, 1,435 grams; 22.5 cc 2 per cent caffein or 314 mg per kilo injected at 11.40 p. m.; 5 a. m., no symptoms.

November 17: Weight, 1,340 grams; 24 cc 2 per cent caffein (358 mg per kilo) injected subcutaneously.

November 18: 9 a. m., rabbit died.

Rabbit 226. Gray male. Diet, carrots.

October 28: Weight, 1,045 grams; 10 cc 2 per cent caffein injected subcutaneously at 1.50 p. m.; 4.30 p. m., tremors observed, but no other symptoms.

October 29: Rabbit in good condition.

November 1: Weight, 950 grams; 10.55 a. m., 11 cc 2 per cent caffein injected subcutaneously (231 mg per kilo).

November 4: Weight, 930 grams; 2.50 p. m., 12 cc 2 per cent caffein injected subcutaneously (258 mg caffein per kilo).

November 6: Weight, 945 grams; 11.45 a. m., 15 cc 2 per cent caffein (313 mg per kilo) injected subcutaneously.

November 17: Rabbit still alive; weight, 890 grams.

The results of these experiments indicate that when sufficient time is allowed between two successive injections, susceptibility to caffein is not increased. The rabbit, on the contrary, seems to acquire a tolerance for the drug, for the fourth dose was 15 per cent larger than the minimum fatal dose of caffein. This is in all probability due to the better elimination of caffein and its products of decomposition and to recovery from the deleterious effects of each dose, made possible by long intervals between injections.

The results of these experiments may be briefly summed up by stating that subminimum doses of caffein given to the rabbit daily or at intervals (not too long) do not produce any symptoms such as were observed in acute caffein intoxication, namely, increased reflexes and convulsions, or increased rate of respiration, thus showing that it is not cumulative. But evidence of undoubted summation of effect was adduced to show that if the administration of subminimum doses of caffein be continued daily for a period of 11 to 18 days the result is fatal. Tolerance, however, may be acquired, although to a limited extent only, provided sufficiently long intervals between injections are allowed to give time for repair of the injury done by the drug and to develop a mechanism for its better decomposition and elimination. Furthermore, the evidence just given indicates that the elimination of subminimum doses of caffein and its products of decomposition is probably accomplished within 24 hours or thereabouts. That the elimination of larger doses is not accomplished in this interval is made probable by the following experiment:

Gray rabbit 455. Female. Diet, oats.

October 12: Weight, 1,185 grams; 3.30 p. m., 11.5 cc 2 of per cent caffein injected into the lumbar muscles; 3 p. m., reflexes increased.

October 13: 10 a. m., rabbit weighed 1,070 grams; no symptoms of caffein poisoning, reflexes normal; 10.30 a. m., 10 cc 2 per cent caffein injected into the lumbar muscles; 11.30 a. m., rabbit jumped off the table, had convulsions, and died.

EXPERIMENTS ON DOGS.

Having gained some information respecting the effects of repeated doses of caffein on rabbits, it was of interest to find out how carnivora reacted to the drug when similarly administered. A number of dogs were used for the purpose. Considerable variation in the mode of experimentation, as will appear later, was allowed.

Since the condition of the animal, its age, environment, or diet might be factors influencing toxicity, tests were made on full-grown and on young growing dogs whose food was varied. The subjects of the experiment were kept under observation for a few days to several weeks before the administration of caffein was begun, in order to determine whether or not any morbid condition existed, as well as to ascertain whether the new environment had any effect on these animals. Caffein was given chiefly by mouth, but the subcutaneous method was also employed during a portion of the experimental period in some dogs. The initial dose, which varied for different individuals, was maintained for a variable length of time. It was then progressively increased, in most cases until the death of the animal. With larger doses the intervals between successive injections were also increased.

SERIES A.

Six dogs were used in this series. Caffein was administered by mouth for periods of six days to five weeks. It was given daily or at intervals of two, and sometimes of three, days. In a few instances the drug was withheld for four or even for seven days, and its administration was resumed at the end of this time. The initial dose in these experiments varied approximately between 40 and 140 mg per kilo. The doses were then increased gradually, and thus the maximum resistance of the subject to caffein was tested. The diet consisted either exclusively of meat or largely of carbohydrates with a minimum amount of meat to give flavor to the food.

Dog 11. Female.

Diet consisted of rice, 250 grams; cane sugar, 250 grams; meat, 50 grams; cracker meal, about 100 grams. Caffein was given by mouth daily or at intervals of one day, when the dose did not exceed 1.5 grams. Before the dose was increased to 2 grams, or approximately 0.213 gram caffein per kilo, an interval of two days was allowed. Symptoms were noticed the next day. An interval of two days was therefore allowed again at the end of which the same dose was repeated. It will be remarked that there were no symptoms this time, and the general condition of the dog seemed to be good. Two grams of caffein were, therefore, given daily during the next two days without any untoward effects; the dose was then increased to 2.5 grams. Even after this enormous quantity no symptoms were observed except slight tremors. When this dose was repeated 26 hours later, it proved fatal. No albumin or sugar was found in the urine, although the dog was fed on a very liberal carbohydrate diet. The following is a complete record of the experiment.

April 20: Urine acid, no albumin, no sugar.

April 21: Urine free from sugar.

April 22: Urine free from sugar. 1 gram caffein given in the afternoon.

April 23: 9 a. m., dog was very thirsty, drank a large quantity of water, urine did not reduce Fehling's solution.

April 24: 2.30 p. m., 1 gram caffein, no sugar in urine.

April 25: 1 gram caffein administered.

April 26: Weight, 10.6 kilos, urine collected in the morning, no sugar; 4.10 p. m., 1.5 grams caffein.

April 27: 1.5 grams caffein; 1.30 p. m., diet as before, no sugar in urine.

April 28: Weight, 10.2 kilos, no caffein, no sugar in urine.

April 30: Weight, 10.4 kilos, no sugar in urine; 4.20 p. m., 2 grams caffein.

May 1: Urine examined, sugar absent, weight 10 kilos, vomited, sick, tremors observed, drank 500 cc water at one time, appetite poor.

May 2: No caffein, drank 150 cc water.

May 3: Urine, no sugar, moderate quantity of albumen present; 12 noon, 2 grams caffein given by mouth, weight 10.3 kilos; 2 p. m., urine, sugar negative, condition of dog good, no symptoms of caffein intoxication.

May 4: 10 a. m., about 10 cc thick, dark-colored mucilaginous urine found in collecting bottle; albumin a little more than a trace, decidedly less than on May 3, no sugar, condition of dog pretty good except for slight muscular tremors; 4 p. m., 2 grams caffein by mouth (as usual).

May 5: Urine not examined, no symptoms; 4 p. m., 2 grams caffein.

May 6: Urine not examined; 2.30 p. m., 2.5 grams caffein given by mouth; 4 p. m., slight tremor, no other symptoms.

May 7: No examination of urine, no symptoms observed; 4 p. m., 2.5 grams caffein.

May 8: 9 a. m., found dead, urine collected since last dose of caffein was given did not contain any sugar or albumin, the amount of caffein fed to this dog was 18 grams in 18 days. *Autopsy:* Post-mortem examination showed marked enteritis with hemorrhagic spots on the mucosa; liver and kidneys congested and dark colored; lungs congested; thyroid gland was greatly enlarged and congested.

Dog 23:

April 30: Weight, 6.8 kilos; the diet consisted of 250 grams rice, 250 grams sugar, 100 grams cracker meal, and 100 grams of meat. On May 3 his weight was 7 kilos. He received 1 gram of caffein by mouth at 12 noon. At 2 p. m. he vomited and tremors were observed. The next day, May 4, tremors were still present though less pronounced. Examination of the urine for sugar and albumin was negative; on May 4, 1 gram caffein was given again and repeated on May 5. On this date his general condition was not good—dog had no appetite and refused to take caffein. As the dog lost 10 per cent of his weight he was put on a meat diet exclusively and the dose of caffein was reduced to 0.5 gram. He became sick after the second dose, and the administration of caffein was therefore discontinued. It was resumed after five days and the caffein was administered in increasing amounts, i. e., on May 18, 0.5 gram; May 19, 0.5; May 20, 1; May 21, 1 gram in two doses of 0.5 each, given at intervals of one hour; May 22, 1 gram. Dog became irritable, but no other symptoms were observed. The administration of caffein was omitted the next day. On the following day when the same dose of caffein was given there was again marked irritability and tremors. The experiment was therefore discontinued.

Dog 22. Male bulldog.

June 24: Dog weighed 13.7 kilos. Diet consisted of meat exclusively; 1 gram caffein was given by mouth; diarrhea developed; no caffein was given for three days.

June 28: Dog weighed 13.6 kilos, 1.5 grams caffein given at 10 a. m.

June 30: 1.75 grams caffein administered.

July 2: Dog weighed 13.5 kilos; 2 grams caffein or 0.15 gram per kilo, caused well-marked thirst, but did not produce any other symptoms.

Dog 20. Female:

May 12: Weight, 7.7 kilos. Fed liberal carbohydrate diet, consisting of rice, 100 grams; sugar 100 grams; meat and cracker meal, a sufficient quantity to flavor the food.

May 14: Weight, 7.7 kilos. Examination of urine for albumin and sugar gave negative results. Urine was acid to litmus.

May 17: Weight, 7.4 kilos. Three hours after it was fed the dog received 0.5 gram caffein by mouth. The test of the urine the next day for sugar was negative, but a trace of albumin was present. It will be noticed that the doses were increased gradually and that symptoms were observed only after the fourth dose of 0.1 gram per kilo. Later meat was substituted for the carbohydrate diet and the administration of caffein was stopped for four days. At the end of this period 100 mg caffein per kilo was fed daily for five days, and the dose was then very gradually increased. Diarrhea occurred twice, but no other symptoms, the second attack having lasted a few days. The following is a complete record of the experiment:

May 19: 0.5 gram caffein 11.45 a. m.
May 20: 0.75 gram caffein 12.45 a. m.
May 21: 0.75 gram caffein 12 noon; no sugar, no albumin in urine.
May 22: 0.75 gram caffein; urine, same condition found; no symptoms.
May 23: Weight, 7.5 kilos; no caffein.
May 24: 0.75 gram caffein; tremors very marked.
May 25: No caffein.
May 26: 0.75 gram caffein.
May 27: 0.75 gram caffein.
May 28: 0.75 gram caffein.
May 29: 1 gram caffein in two doses of 0.75 and 0.25 gram.
May 30: No caffein.
May 31: No caffein; meat diet exclusively.
June 1: No caffein; meat diet exclusively.
June 2: No caffein; no sugar, no albumin in urine.
June 3: Weight, 7.6 kilos; 0.75 gram caffein; no sugar in urine.
June 4: Weight, 7.3 kilos; 0.75 gram caffein; no sugar in urine.
June 5: Weight, 7.5 kilos; 0.8 gram caffein; drank 500 cc water; ate 200 grams meat.
June 6: Weight, 7.4 kilos; 0.8 gram caffein; 500 cc urine; drank 500 cc water; ate 200 grams meat; no symptoms.
June 7: Weight, 7.7 kilos; 0.8 gram caffein 10 a. m.; 400 cc urine, 500 cc water, 200 grams meat.

June 8: Weight, 7.5 kilos; 0.9 gram caffein, 450 cc urine, 1 p. m.; 200 grams meat, 500 cc water.

June 9: Weight, 7.6 kilos; 0.9 gram caffein, 1 p. m.; 500 cc water, 200 grams meat and bone dust; diarrhea and restlessness all afternoon.

June 10: Weight, 7.6 kilos; 1 gram caffein, 500 cc water, 200 grams meat, 480 cc urine.

June 11: Weight, 8 kilos; 1 gram caffein, 470 cc urine, 500 cc water, 200 grams meat.

June 12: Weight, 7.8 kilos; 1 gram caffein, 710 cc urine, 500 cc water, 200 grams meat.

June 13: 450 cc urine, 500 cc water, 300 grams meat.

June 14: Weight, 7.9 kilos; 1.2 grams caffein, 500 cc water, 300 grams meat, 490 cc urine.

June 15: Weight 7.8 kilos, 500 cc water, 300 grams meat, 550 cc urine.

June 16: Weight 8.0 kilos, 1.2 gram caffein, 500 cc water, 300 grams meat, bone dust added to check diarrhea.

June 17: 500 cc water, 300 grams meat, 450 cc urine, diarrhea continues, bone dust added.

June 18. Weight 7.8 kilos, 1.3 gram caffein, 300 grams meat, 500 cc water, 300 cc urine.

June 19: Dog very thirsty, drank 1 liter of water and ate 350 grams of meat; 960 cc urine passed during the past 24 hour.

June 21: Weight 7.5 kilos, 1.5 grams caffein given at 10 a. m. At 2 p. m. convulsions and death. This dog received a total of 21.15 grams caffein in 25 doses during a period of 35 days, which amounts to an average of 85 mg per kilo daily.

Dog 19. Female fox terrier.

May 13: Weight 6.4 kilos. Diet consisted of rice, 100 grams; sugar, 100 grams; and a sufficient quantity of meat and cracker meal to give flavor to the food. Examination of the urine showed a trace of albumin but no sugar. The urine was acid to litmus. Two days later the urine was alkaline to litmus. There was still a small amount of albumin but no sugar.

May 17, 0.5 gram caffein was given by mouth. Examination of the urine collected the next day still showed the presence of albumin and the absence of reducing substances. The dog had tremors. Caffein was, therefore, not administered.

May 19: 0.5 gram caffein was given by mouth.

May 20: 0.75 gram caffein was fed at 12.45 p. m. The dog vomited during the night and tremors were observed the next morning. The urine collected was examined for albumin and sugar, but neither was found.

May 21: 12 noon, 0.75 gram caffein was fed. The dog weighed 6 kilos, which therefore represented a loss of 0.4 kilo. Grew abnormally thirsty and lost appetite, but no other symptoms of caffein poisoning were observed.

May 22: The dog was again given 0.75 gram caffein at 12 noon. The examination of the urine for albumin and sugar gave negative results. The dog died at 4.15 p. m. The fatal dose for this dog was therefore 0.125 gram caffein per kilo, and the total amount of caffein ingested in six days amounted to 3.25 grams, or 0.54 gram per day, which makes 90 mg per kilo.

Dog 21. White female bull.

This dog was kept on a diet exclusively of meat, and was given water ab libitum. From 0.5 to 0.6 gram of caffein was administered daily for seven days; the doses were then increased and were given at longer intervals. No symptoms of the effects of caffein were observed until a dose of 1.5 gram was fed, when diarrhea was noticed on the next day. In the following record the details of the experiment are given:

Date.	Weight.	Caffein.	Date.	Weight.	Caffein.
	Kilos.	*Grams.*		*Kilos.*	*Grams.*
June 7	12.5	0.5	June 16	12.7	0.8
June 8	12.5	.5	June 18	12.9	1.0
June 9	12.5	.5	June 21	13.4	1.2
June 10	12.3	.6	June 24	13.3	1.5
June 11	12.3	.6	June 25	(1)	.0
June 12	12.3	.6	June 27	13.5	1.5
June 13	12.3	.6	June 30	13.5	1.75
June 14	12.3	.8			

[1] Diarrhea.

July 2: 11.30 a. m., 2.0 gram caffein fed by mouth; 1.30.p. m., tetanus, dog died. The total amount of caffein fed to dog No. 21 out of the 25 days of the experiment was 14.45 grams, or an average of 578 mg per day, which amounts to about 42 to 43 mg per kilo of body weight.

Notwithstanding the diversity in the method of experimentation, there was a striking uniformity in some of the results obtained. All the experiments of the series showed absence of cumulative action of caffein. The experimental evidence presented indicates that moderately large doses may be given at intervals of about 24 hours without inducing any symptoms of nervous or any other disturbance. This is illustrated in the tests on dog 11, which were preliminary in character. In this subject 100 to 150 mg of caffein per kilo were ingested daily for several days without showing any changes. Later in the course of the experiment, after larger doses were given, mild symptoms only, such as tremors, were observed. Additional evidence of the absence of cumulative action of caffein was furnished by the results of the following experiments:

Dog 23 received 142 mg of caffein per kilo on three successive days. His general condition indicated that these amounts of caffein were toxic, but he survived. In another series of tests, made after he was allowed to rest a few days, he again failed to show any cumulation of the drug, as he survived this time a series of tests of longer duration than the first.

A much better illustration of the absence of cumulative action of the drug is furnished by the experiments on dog No. 20. In this case 100 to 125 mg of caffein per kilo, given on 10 consecutive days, did not cause any marked effects. Diarrhea and restlessness were the only symptoms observed. These experiments therefore show that the elimination and decomposition of caffein are apparently effected by the body within twenty-four hours or thereabouts.

Experiments on dog 19, however, form an exception—the third dose of 125 mg caffein per kilo having proved fatal. The very low protein content of the diet of this dog suggests itself as a possible cause of the lower resistance to caffein of this subject. But it may be observed that the same diet was furnished to dog 20, which stood such amounts of caffein much longer. The presence of a trace of albumin in the urine of dog 19 is likewise inadmissible as a cause of the difference in the toxicity of caffein in this dog, for the urine of dog 20 likewise contained a trace of albumin. The alkaline reaction of the urine, together with the fact that the first dose of only 60 mg of caffein per kilo induced symptoms of toxicity, suggests the presence of an abnormal condition which in all probability was the cause of the death of this subject under the conditions indicated.

In a large number of experiments on caffein performed in this laboratory it has been observed that symptoms due to caffein often disappeared when the administration of the same dose of the drug was continued. Thus dog 19 vomited when the amount of caffein was increased to 125 mg per kilo. When this amount was repeated the next day there was no vomiting. Similar observations were made on dogs 11 and 23, also on other dogs. No. 22 developed diarrhea at first; when the administration of caffein was resumed several days later, however, there was no diarrhea. In other experiments performed in this laboratory, symptoms of nervous irritability induced by caffein disappeared on continued treatment.

It was interesting, therefore, to inquire whether resistance to caffein would be increased by the continued administration of progressively larger amounts of the drug. When doses of 150 and over were fed, the intervals allowed were usually longer than 24 hours. Two and sometimes three days were permitted to elapse between two successive doses. This was done in order to allow time for recovery from possible changes induced by larger doses of caffein, and thus prevent the summation of effect. In the experiments considered, therefore, Nos. 11, 23, 20, and 19, the toxicity of caffein does not seem to be greater than in the experiments on acute caffein intoxication in the dog. It was thought, however, that the large initial doses or the quick change to large doses when the amounts used in the beginning were small, might have something to do with failure to induce a marked degree of tolerance. The experiment on dog 21 was therefore carried out by giving from 40 to 60 mg per kilo for eight days, and then increasing the dose, but tolerance could not be induced, as is shown in the protocol to the experiment.

SERIES B.

According to the studies of Chittenden,[16] low protein diet improves the general metabolism of the body, fatigue is diminished, and bodily vigor, therefore, correspondingly increased. The expectation is, therefore, justified that the defense of the organism against deleterious substances introduced into the body is much improved by such a diet, thus increasing its resistance to poisons. Hunt's experiment on this subject, also quoted by Chittenden, lends support to this view. He found that mice fed on carbohydrates chiefly, or on foods containing only a small amount of protein, were more resistant to acetonitril. It was interesting, therefore, to inquire whether the toxicity of caffein differs under similar conditions of diet.

A fixed diet of the same calorific value was provided for all dogs of this series, but the protein content for three of the animals was approximately one-third of the amount usually fed to dogs. Caffein was at first administered subcutaneously, but all the dogs on a low protein diet developed abscesses at the site of injection, while none of those on high protein diet showed a local reaction. Feeding by mouth was then begun and continued throughout the experiment in each case. The initial dose was 50 mg per kilo, which was given daily for seven to nine days. It was then increased progressively by 25 mg per kilo; 75 mg per kilo were administered for one to two days, 100 mg for two to three days, 125 mg for one to two days, 150 mg for one to two days, and a single dose of 175 mg. It will be remarked that sometimes an interval of one day had to be allowed during which no caffein was fed.

Dog 30. Black and tan hound, male.

The dog was under observation for about eight weeks before the experiment was begun and had received a high protein diet. He was then given 50 mg caffein for nine consecutive days. On the tenth day the dose was increased to 75 mg per kilo. As no symptoms developed, this dose was increased to 100 mg per kilo, and was fed one day apart. It was then raised to 125 mg per kilo. For the first time since the drug was fed, symptoms appeared; they were noticed a few hours after feeding and persisted during the next day. Although the appetite was good, no caffein was given on this day. On the following day this dose was repeated. As the symptoms were not serious, 150 mg per kilo were given daily for the next three days, until 175 mg per kilo was reached. This dose proved fatal within six hours. Record of experiment follows:

October 9: Weight, 9 kilos, on full nitrogen diet, received daily 0.724 gram nitrogen per kilo or 87 calories per kilo, received 18 grams meat per kilo, 4 grams lard per kilo, 3 grams carbohydrates per kilo, bone dust, ad libitum.

November 3: Weight, 9.10 kilos.

November 10: Weight, 9 kilos.

November 20: Weight, 9.55 kilos.

November 29: Weight, 8.70 kilos.

December 6, 7, 8, and 9: Received subcutaneously 22 cc 2 per cent caffein. Condition good, site of injection normal.

December 10, 11, 12, 13, and 14: Received 0.4375 gram caffein by mouth equal to 0.050 gram per kilo, no symptoms, appetite and general condition good.

December 15: 11.30 a. m., received 0.6563 gram caffein by mouth, or 0.75 gram per kilo, no symptoms, appetite good, condition excellent.

December 16: 11 a. m., received 0.870 gram caffein by mouth, or 0.1 gram per kilo, weight 8.70 kilos, no symptoms.

December 17: No caffein given.

December 18: Received 0.870 gram caffein, or 0.1 gram per kilo, no symptoms.

December 20: 2.45 p. m., received 1.0875 grams caffein, or 0.125 per kilo; 4 p. m., ate food readily, seemed very uncomfortable and sick.

December 21: 9 a. m., stiffness in muscles, but no other symptoms, appetite good, no caffein given.

December 22: 11 a. m., received 1.0875 grams caffein, or 0.125 gram per kilo; 3 p. m., depressed in spirits and sick, but no other symptoms observed.

December 23: 11.30 a. m., received 1.305 grams caffein, or 0.150 gram caffein per kilo; 1.30 p. m., apparently quite sick, but no other symptoms, had good appetite.

December 24: 10 a. m., received 0.175 gram caffein per kilo; 4 p. m., when about to be fed fell over and died; no autopsy.

The total amount of caffein given dog 30 was 11.3458 grams, administered for a period of eighteen days. The average daily amount per kilo was therefore 72 mg. The feces became offensive when the amounts of caffein were increased to 75 mg per kilo. It will be observed that in this dog the appetite was uniformly good until the day of his death. Whether or not this is the cause of his resistance to caffein will be discussed later.

Dog 32. White, male, young.

Although he was growing rapidly this dog's weight was constant, but he looked anemic. He received a high protein diet until December 3, when the rations were increased by one-third. This dog was under observation from October 26 to December 6 when the administration of caffein was begun. He then received 50 mg caffein per kilo daily for nine days consecutively without showing any effects, when the dose was increased to 75 mg per kilo, then to 100 mg per kilo. This dose was further increased to 150 mg per kilo without causing symptoms, which was repeated the next day. No symptoms having been observed after such amounts of caffein, 175 mg per kilo were fed. This dose, however, proved fatal within two hours. Record of experiment follows:

October 26: Weight, 6.90 kilos.

November 3: Weight, 6.90 kilos.

November 10: Weight, 6.90 kilos.

November 20: Weight, 6.90 kilos.

November 29: Weight, 6.55 kilos.

December 3: Put into cage, diet increased one-third.

December 6, 7, 8, 9: Weight 6.30 kilos; 12.30 p. m., received 16 cc 2 per cent caffein by subcutaneous injection in back, no symptoms of any kind noticed, site of injection normal.

December 10, 14: 0.05 gram caffein per kilo.

December 15: Received 0.4725 gram caffein by mouth, no symptoms.

December 16: Received 0.655 gram caffein, 0.100 gram per kilo.

December 17: No caffein given.

December 18: Received 0.655 gram caffein daily, 0.100 gram per kilo, no symptoms.

December 20: Received 0.8188 gram caffein, 0.125 gram per kilo, no symptoms, appetite good.

December 21: Received 0.9825 gram caffein, 0.150 gram per kilo, somewhat uncomfortable, no other symptoms.

December 22: Received 0.9825 gram caffein, 0.150 gram per kilo, no symptoms except some uneasiness.

December 23: 9 a. m., no symptoms, appetite good; 11.30 a. m., received 1.146 grams caffein, 0.1759 gram per kilo; 1.30 p. m., died while making an effort to get out of cage, tonic contraction of limbs observed before death.

The amount of caffein received during the entire experimental period was 9.2223 grams, or an average per day approximately of 80 mg per kilo, and therefore 10 per cent more than dog No. 30 received. It will be observed that the appetite in dog No. 32 was likewise uniformly good, and that he received a very high protein diet which was also of a very high calorific value.

Autopsy (*dog 32*).—Stomach presented a severe inflammation of the mucosa, especially in the fundus and pyloric portions. The gastritis was more marked in pyloric portion, and the inflammatory condition extended along the whole course of small intestines, which presented numerous hemorrhagic areas, and a thick catarrhal exudate on the mucosa. The large intestine contained quite a large number of parasites, probably round worms. The liver was enlarged and the gall cyst well filled. The spleen was also considerably engorged, kidneys appeared normal, other organs all appeared normal.

Dog 31. Black spaniel, male.

This dog had been under observation one month previous to the experiments with caffein. The usual initial dose was then administered for nine days. There were no signs of local irritation when the drug was given subcutaneously, but symptoms of toxicity were present. These disappeared, however, when the drug was administered by mouth. The dose was therefore increased to 75 mg per kilo. This, as will be seen, proved fatal within six hours. High nitrogen diet, same as No. 30.

November 3: Weight 10.250 kilos.

November 10: Weight, 10.25 kilos.

November 20: Weight, 10.30 kilos.

December 1: Put in cage.

December 6, 7, 8, 9: Weight, 10.20 kilos; received 26 cc 2 per cent caffein subcutaneously, site of injection normal.

December 6: Very restless and excited, whined when handled as though muscles were sore, appeared to be sick.

December 10–14: Condition good, received 0.51 gram caffein by mouth daily, no noteworthy symptoms, appetite continues good, somewhat restless at intervals.

December 15: 11.30 a. m., received 0.765 gram caffein per mouth (0.075 gram per kilo); 2 p. m., depressed in spirit, seemed sick and uncomfortable; 4.15 p. m., when about to feed, animal jumped up, then fell back dead.

Autopsy (*dog 31*): Lungs congested; heart filled with blood and contained small amount of blood-stained fluid in pericardial sac. Liver deeply congested, soft and friable; gall bladder distended with bile; kidneys showed inflammation of cortex; spleen pale, normal in size and consistency; stomach practically empty, the mucosa of the pyloric portion exhibited severe gastritis, with thick catarrhal exudate. This catarrhal inflammation extended through the duodenum; remaining portion of small intestine showed mild inflammation; large intestine appeared practically normal. The total amount of caffein received by dog 31 during 10 days was 5.395 mg, or a daily average of 53.9 mg per kilo. This unusually low resistance to caffein (which was practically the only case in all the experiments on dogs presented in this research) suggests the presence of some abnormal condition. The bloody exudate in the pericardial cavity indicating pericarditis, which is likely to induce secondary changes of cardiac muscle, may be considered as a possible cause of the increased toxicity of caffein in this case.

Dog 29. Male fox terrier, black.

This dog was kept on a low nitrogen diet for nearly five weeks before the feeding of caffein was begun. The administration of 50 mg of caffein per kilo was then carried on for eight days without showing any symptoms of toxicity. The usual increase of dose was then given—75 mg per kilo—which was followed by a manifestation of symptoms. Further increase, however, to 100 mg per kilo had no visible effect. Nevertheless it was considered advisable to suspend the feeding of caffein for one day. The same amounts were then repeated on two consecutive days. No symptoms having been observed, 125 mg per kilo were given. As symptoms of toxicity and especially loss of appetite were observed, the dog was not given any caffein the next day. Since his appetite had now improved, the experiment with larger doses was resumed. Death followed after the second dose of 150 mg per kilo. Protocol follows:

Weight, 9.90 kilos. One-third nitrogen diet. Receives 0.269 gram nitrogen per kilo (88.269 calories per kilo).

November 3: Weight, 9.85 kilos.

November 10: Weight, 9.55 kilos.

November 12: Weight, 9.40 kilos.

November 29: Weight, 9.85 kilos.

December 6: Weight, 9.90 kilos; 11.35 a. m., received 25 cc 2 per cent caffein solution by subcutaneous injection in back; 4 p. m., no symptoms, appetite good.

December 7–9: Received 25 cc caffein 2 per cent solution—subcutaneous injection, no symptoms, area of injection inflamed and swollen.

December 10, 13: Site of injection showed increased inflammation, received 0.495 gram caffein (50 mg per kilo) in 30 grams meat daily without showing any symptoms.

December 14: 12 noon, received 0.7425 gram caffein by mouth (0.075 per kilo); 2.30 p. m., restless and uneasy.

December 15: 11.30 a. m., received 0.7425 gram caffein by mouth; 2 p. m., depressed in spirits, although continues to have good appetite.

December 16: Weight, 9.50 kilos; 3.15 p. m., received 0.9509 gram caffein by mouth; 4.50 p. m., no symptoms.

December 17: Animal rested.

December 18: Received 0.950 gram caffein by mouth, no symptoms.

December 19: Received 0.9509 gram caffein by mouth, no symptoms.

December 20: 2.45 p. m., received 1.1875 grams caffein (0.125 gram per kilo); 4 p. m., restless and quite sick; ate only a little food.

December 21: 9 a. m., still uncomfortable, allowed to rest, no caffein given, gradually recovered appetite.

December 22: 11 a. m., received 1.875 grams caffein; 3 p. m., seemed sick, but showed no other symptoms, appetite fair.

December 23: 9 a. m., showed no symptoms from the day before, ate food gradually, seemed sick; 11.30 a. m., received 1.425 grams caffein (0.150 gram per kilo); 1.30

p. m., looked and behaved as if very sick, no other symptoms; 3.45 p. m., in attempting to get out of box fell over on back, had convulsions, whined, dyspnœa, died within 30 seconds.

Autopsy: Stomach exhibited mild inflammation; small intestine inflamed and hemorrhagic areas on mucosa; liver engorged and friable; spleen normal; kidneys slightly congested; other organs appeared normal. The total amount of caffein fed to Dog 29 was 12.135 grams, which was given in 18 days. The average daily amount per kilo was therefore 67.68 mg.

Dog 28. Male fox terrier.

Low nitrogen diet was begun about four weeks before the feeding of caffein; 50 mg of caffein was then fed for seven consecutive days. Partial loss of appetite was observed after the first dose. As the experiment progressed the desire for food steadily diminished, and the feces became fetid. Symptoms of intoxication manifested themselves early in the experiment, and vomiting occurred after the fourth dose. The dog was then put on a diet exclusively of meat. After an intermission of 10 days 109 mg caffein per kilo were given. Since there were no symptoms, the following day the amount was increased to 125 mg per kilo. This dose proved fatal within 16 to 20 hours.

This dog was stout and strong, weight 12.25 kilos, received daily 0.269 gram nitrogen per kilo (88.269 calories per kilo).

November 3: Weight, 11.75 kilos.

November 10: Weight, 11.95 kilos.

November 20: Weight, 11.20 kilos. All through this period had been kept in a cold, poorly ventilated room, put in a warm room, with bedding and good ventilation.

November 29: Weight, 11.95 kilos.

December 1: Put in a cage; weight, 11.95 kilos.

December 6: Weight, 11.95 kilos; 11.45 a. m., received 0.050 gram caffein per kilo; then received 30 cc 2 per cent caffein (0.6 gram) in practically one subcutaneous injection; 4.30 p. m., ate only part of food.

December 7: 10.25 a. m., received 30 cc 2 per cent caffein by subcutaneous injection (0.6 gram, or 50 mg, per kilo); 1.45 p. m., seemed sensitive to touch, no desire for food, depressed in spirit.

December 8: 11.40 a. m., received 30 cc 2 per cent caffein by subcutaneous injection (50 mg per kilo); 1 p. m., depressed in spirit, hind legs seemed somewhat stiff, no desire for regular food, site of injection inflamed.

December 9: 10.50 a. m., received 30 cc 2 per cent caffein by subcutaneous injection (50 mg per kilo); 2.30 p. m., had vomited, no desire for regular food.

December 10: Inflammation of site of injection, and swelling very pronounced; 2 p. m., received 0.5975 gram caffein, or 50 mg per kilo, with 30 grams of meat, refused regular food.

December 11, 12: Received 0.5975 gram caffein by mouth, no symptoms except refusal of regular food, feces fetid.

December 13–22: Put on meat diet exclusively, high temperature, no caffein, weight 10 kilos, appetite good, feces fetid.

December 22: 12 m., weight 11 kilos, received 1.2 grams caffein by mouth (0.109 gram per kilo); 4 p. m., no symptoms.

December 23: 11.30 a. m., received 1.375 grams caffein (0.125 gram per kilo) had vomited food of the day before, but could notice no caffein or capsules in vomit; 4.30 p. m., no symptoms, seemed in good spirits, appetite good, had no meat to feed with, so was given low nitrogen feed, of which he ate about one-fourth.

December 24: 9 a. m., found dead, stiff, and cold. The most striking effect of caffein in this dog is the increased intestinal putrefaction. The feces were still fetid 10 days after the administration of caffein was stopped.

Autopsy, dog 28: Stomach partially filled with an undigested food mass; mucosa showed severe inflammation; small intestines presented a hemorrhagic enteritis along whole extent; large intestine also exhibited mild inflammation; liver was engorged; spleen appeared normal; kidneys slightly congested in cortical portion; other organs appeared normal.

Dog 24. White and tan male: Was put on low protein diet six weeks before experiments with caffein were begun. The initial dose of 50 mg per kilo was then administered on eight consecutive days. The only symptoms observed during this period of caffein administration were those of intestinal putrefaction. Fetid feces were noticed already after the first dose of caffein was injected. When the second dose of 75 mg of caffein was repeated, mild symptoms appeared, but none have been observed even with increased amounts of caffein.

One-third nitrogen diet. Received daily 0.269 gram nitrogen per kilo (88.269 calories per kilo).

October 26: Weight 11.15 kilos. Food consisted of 5 grams cracker meal per kilo; meat, 3 grams per kilo; lard, 2 grams per kilo; tapioca, 10.69 grams per kilo. Kept in a cold, damp room with poor ventilation until November 20.

November 3: Weight, 11 kilos.

November 10: Weight, 10.75 kilos.

November 20: Weight, 10.55 kilos; changed to a warm room, with bedding and good ventilation.

November 29: Weight, 10.85 kilos.

December 1: Put into a cage.

December 6: Weight, 10.90 kilos; 11.25 a. m., received 28 cc 2 per cent caffein subcutaneously in side, below the shoulders, area washed with alcohol and ether, approximately 50 mg per kilo administered, no symptoms.

December 7: 10.15 a. m., received 28 cc 2 per cent caffein injected subcutaneously; feces soft and very fetid; 1 p. m., depressed in spirit, eyes dull.

December 9: 10.45 a. m., received 25 cc 2 per cent caffein solution subcutaneously, feces still fetid, site of injection inflamed and swollen, no other symptoms.

December 10: Inflammation of area of injection more pronounced; 2 p. m., given 0.5449 gram caffein and 30 grams of meat; 4 p. m., fed, no symptoms, feces fetid.

December 11: 12 m., given 0.5459 gram caffein and 30 grams of meat, no symptoms, feces fetid.

December 12, 13: Given 0.5459 gram caffein daily, without noticing any symptoms.

December 14: 12 m., received 0.817 gram caffein (75 mg per kilo); 2.30 p. m., restless and uncomfortable, no other symptoms.

December 15: 11.30 a. m., received 0.8175 gram caffein by mouth; 2 p. m., depressed in spirit, acted as though sick, no other symptoms.

December 16: Weight, 11 kilos; 11 a. m., received 0.100 gram caffein per kilo (1.100 grams) by mouth, no symptoms.

December 17: Rested.

December 18: 2.30 p. m., received 1.100 grams caffein by mouth; 4. p. m., no symptoms.

December 19: 12 noon, received 1.100 grams caffein by mouth; 4.15 p. m., no symptoms.

December 20: 2.45 p. m., given 1.375 grams caffein (0.125 gram per kilo); 3.45 p. m., vomited—one of the capsules being found intact, the other broken open; 4 p. m., given regular diet, containing 1.3757 grams caffein in capsules, ate most of this during the night, whined at intervals, coordination disturbed, appeared very sick, but exhibited no other symptoms.

December 21: 9 a. m., found dead, stiff, and cold.

The total amount of caffein received by dog 24 was between 10.109 and 11.484 grams. As one of the capsules vomited was intact and the other broken open, the amount was probably about 10.75 grams. The fatal dose in this case was undoubtedly less than 185 mg per kilo—somewhere between 125 and 185 mg. Autopsy showed heart in diastole; posterior lobe of right lung deeply congested; liver engorged; gall cyst filled; spleen appeared normal; stomach well filled with semifluid mass; pyloric portion of stomach exhibited a severe inflammation of mucosa; mucosa of duodenum greatly inflamed and showed hemorrhagic areas and catarrhal exudate; remainder of small intestine also exhibited mild inflammation; kidneys deeply engorged, mesentery injected.

A comparison of the fatal doses of caffein in the experiments on high and low protein diet does not show much difference in the resistance to caffein, since 175 mg per kilo proved fatal to Nos. 30 and 32, while No. 29 died after receiving 150 mg per kilo, and No. 24 received 125 to 185 mg per kilo. Moreover, No. 28, which was changed from low to high protein diet, succumbed when given 125 mg per kilo. Observations made during the experimental period indicate, however, greater toxicity of caffein in the subjects on low protein diet. Dog 30 showed the effects of the drug when the dose was increased to 125 mg of caffein per kilo, while in No. 32, 150 mg per kilo were received without any manifestation of symptoms. Dog 31, which was likewise on a high protein diet, is evidently an exception, and its low resistance to caffein may be accounted for by the condition found at autopsy. In other dogs on low protein diet symptoms of intoxication appeared early in the experiment. In Nos. 29 and 24 it was observed as soon as the amount of caffein was increased to 75 mg per kilo. In dog 28 the first dose of caffein 50 mg per kilo was toxic. The symptoms of gastro-intestinal disturbance were especially marked after caffein on low protein diet. This may seem to contradict the results of experiments on dogs 11 and 20, in which larger doses of caffein failed to induce symptoms of intoxication. But it should be observed that the diet, which consisted almost exclusively of carbohydrates, was given only during the administration of caffein, while in the experiments of series B the subjects received a low protein diet for several weeks before the administration of caffein was begun, and it was continued through the entire caffein period. It will be remarked that the absence of cumulative action in the experiments of the preceding series was also observed in dogs on high as well as on low protein diet. The appearance of symptoms after smaller doses of caffein in the latter experiments might suggest cumulative action, but since these symptoms disappeared on continued administration of the substance cumulation is clearly not indicated. The gastrointestinal lesions observed on post-mortem examination were, it will be recalled, also found in rabbits similarly treated. The explanation suggested probably applies also in the case of dogs.

SERIES C.

As already pointed out in the experiments on acute toxicity of caffein, young growing dogs are probably more resistant to caffein than adults. That this may also hold true in chronic caffein intoxication seemed indicated by the following experiments.

Dog 33. Black female puppy. Weight, 4 kilos. Had been continuously on a meat diet.

December 22: 2.30 p. m., received 0.69 gram of caffein (0.172 gram per kilo); 3.15 p. m., no symptoms except that feces were fetid.

December 23: 11.30 a. m., received 0.79 gram of caffein (0.197 gram per kilo); 1.30 p. m., no symptoms.

December 24: 11 a. m., received 0.87 gram of caffein (0.2009 gram per kilo); 4 p. m., no symptoms.

It will be observed that the only effect produced in dog 33 by feeding caffein was increased intestinal putrefaction, although 2.37 grams of caffein were given in three days. Additional data on the effects of the age of animals on the resistance to caffein seemed desirable. The following experiments were therefore carried out. Six puppies of the same litter were weaned when 7 to 8 weeks old and put on a milk diet. Three of them received this diet throughout the experimental period. Meat was substituted in the other three a few days before the administration of caffein was begun, and was continued until the end of the experiment. Caffein was given by mouth; the initial dose, which was administered for several days and then gradually increased, being 160 to 200 mg for each dog, except one, which received only 100 mg per kilo for several days and then an increased amount.

An intermission of a few days (during which no caffein was given) was allowed. This was done on account of some studies carried on at the same time on the effect of caffein on certain constituents of the urine.

PUP NO. 1.

Date.	Weight.	Food (milk).	Treatment (2 per cent caffein).	Symptoms.
	Grams.	*cc.*	*cc.*	
Apr. 21	1,450	300	10.0	No symptoms.
Apr. 22	1,520	300	10.0	Do.
Apr. 23	1,450	250	10.0	Do.
Apr. 24	1,375	250	10.0	Do.
Apr. 25	1,420	250	10.0	Do.
Apr. 26	1,390	250	None.	
Apr. 27	1,400	250	None.	
Apr. 28	1,405	250	None.	
Apr. 29	1,420	250	None.	Passed worms.
Apr. 30	1,430	250	None.	Do.
May 1	1,450	250	10.0	No symptoms.
May 2	1,515	250	15.0	Do.
May 3	1,475	250	15.0	Do.
May 4	1,495	250	15.0	Do.
May 5	1,515	250	22.0	Seems dull and whines.
May 6	1,535	250	20.0	Whines.
May 7	1,525	250	20.0	No symptoms.
May 8	1,530	250	20.0	Do.
May 9	1,500	250	23.0	Diarrhea; passed worms; tremor and rigidity of legs; whines.
May 10	1,490	250	None.	Completely recovered from the effects of 9th.
May 11	1,535	250	25.0	Can not balance itself; continually vomiting.
May 12	1,460	300	None.	Recovered from effects.
May 13	1,475	350	None.	In good condition.
May 14	1,545	250	None.	
May 15	1,550	250	None.	
May 16	1,555	250	None.	
May 17	1,560	250	25.0	Salivated in cage; stiffness of muscles.
May 18	1,450	250	None.	Weak and stiff; diarrhea.
May 19	1,500	250	None.	No symptoms.
May 20	1,565	250	None.	
May 21	1,545	250	None.	
May 22	(1)	250	None.	
May 23	1,595	250	27.0	Tremors; gait clumsy; incoordination of movements.
May 24	1,495	250	27.0	Diarrhea; vomited; weak and stiff; found dead 9 a. m. 25th.

[1] Sunday.

Autopsy: Marked pulmonary congestion; liver very pale; heart wall injected; slight inflammation of stomach and intestines.

PUP NO. 2.

Date.	Weight.	Food (milk).	Treatment (2 per cent caffein).	Symptoms.
	Grams.	*cc.*	*cc.*	
Apr. 21	1,350	300	5.0	No symptoms.
Apr. 22	1,240	300	5.0	Do.
Apr. 23	1,250	200	7.5	Do.
Apr. 24	1,205	200	7.0	Do.
Apr. 25	1,220	200	7.0	Do.
Apr. 26	1,210	200	None.	
Apr. 27	1,210	200	None.	
Apr. 28	1,205	200	None.	
Apr. 29	1,200	200	None.	Passed worms.
Apr. 30	1,210	200	None.	
May 1	1,220	200	10.0	No symptoms.
May 2	1,220	200	10.0	Do.
May 3	1,235	200	10.0	Do.
May 4	1,235	200	10.0	Do.
May 5	1,235	200	17.0	Whines.
May 6	1,250	200	17.0	Do.
May 7	1,235	200	15.0	Diarrhea and worms.
May 8	1,250	200	15.0	Diarrhea.
May 9	1,165	200	18.0	Little or no symptoms.
May 10	1,235	200	None.	No symptoms.
May 11	1,300	200	20.0	Salivated in cage; refused to eat; draws up hind legs.
May 12	1,200	200	None.	Recovered.
May 13	1,215	200	None.	In good condition.
May 14	1,280	200	None.	
May 15	1,300	200	None.	
May 16	1,310	200	None.	
May 17	1,310	200	20.0	Salivated in cage; stiff; all symptoms.
May 18	1,250	200	None.	Weak and stiff.
May 19	1,245	200	None.	No symptoms.
May 20	1,310	200	None.	
May 21	1,325	200	None.	
May 22	1,325	200	None.	
May 23	1,325	200	22.0	Somewhat stiff.
May 24	1,315	200	22.0	Restless; scratches eyes; sick.

PUP NO. 3.

Apr. 21	1,215	300	None.	
Apr. 22	1,220	300	None.	
Apr. 23	1,220	200	None.	
Apr. 24	1,200	200	None.	
Apr. 25	1,205	200	None.	
Apr. 26	1,195	200	None.	
Apr. 27	1,200	200	(1)	
Apr. 28	1,215	200	None.	
Apr. 29	1,220	200	None.	
Apr. 30	1,200	200	None.	
May 1	1,225	200	10.0	No symptoms.
May 2	1,230	200	10.0	Do.
May 3	1,235	200	10.0	Coughs and whines.
May 4	1,245	200	10.0	Passed worms.
May 5	1,270	200	17.0	Eyes appear dim and is continually scratching them.
May 6	1,260	200	17.0	Appears restless and draws up hind legs when walking.
May 7	1,240	200	15.0	Eyes dim; passed worms; diarrhea.
May 8	1,265	200	15.0	Coughing continually; very restless.
May 9	1,240	200	18.0	12 noon; salivated in cage; passed worms; diarrhea; foaming at mouth; can not balance himself; rigidity and tremor of hind legs. 2.15, found dead.

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[1] Urine squeezed from bladder.

Autopsy: Severe pulmonary congestion; catarrhal gastritis; mild enteritis with small hemorrhagic areas on mucosa.

PUP NO. 4.

Date.	Weight.	Food.	Treatment (2 per cent caffein).	Symptoms.
	Grams.	*Milk* (cc).	cc.	
Apr. 28	1,670	300	....	
Apr. 29	1,670	300	....	
Apr. 30	1,670	300	....	
May 1	1,690	300	....	
May 2	1,690	300	....	
May 3	....	300	....	
May 4	1,720	300	....	
May 5	1,735	300	....	
		Meat (*grams*).		
May 6	1,760	60	....	
May 7	1,745	80	....	
May 8	1,710	180	....	
May 9	1,750	180	....	
May 10	1,750	180	....	
May 11	1,755	180	10.0	No symptoms.
May 12	1,730	180	None.	
May 13	1,785	180	None.	
May 14	1,835	115	10.0	Do.
May 15	1,820	115	10.0	Do.
May 16	1,835	115	10.0	Passed worms.
May 17	1,860	115	10.0	Feces soft and black.
May 18	1,855	115	15.0	Stiff; loss of appetite.
May 19	1,770	115	15.0	Loss of appetite.
May 20	1,755	115	15.0	Do.
May 21	1,780	115	17.0	Restless.
May 22	....	115	17.0	Feces soft and black.
May 23	1,785	115	17.0	Loss of appetite.
May 24	1,795	115	....	Loss of appetite; threw up worms.
May 25	1,630	115	20.0	Loss of appetite; worms; cough; diarrhea.
May 26	1,600	115	23.0	Weak; no appetite; diarrhea; cough.
May 27	....	....	....	Found dead, 9 a. m.

Autopsy.—Lung uniformly congested; liver deeply congested; heart muscle pale with hemorrhagic areas; kidneys pale with hemorrhagic spots on surface and in cortex; slight catarrhal inflammation of stomach and the small intestines.

PUP NO. 5.

Date.	Weight.	Food.	Treatment (2 per cent caffein).	Symptoms.
	Grams.	*Milk* (cc).	cc.	
Apr. 28	1,745	300	....	
Apr. 29	1,745	300	....	
Apr. 30	1,750	300	....	
May 1	1,765	300	....	
May 2	1,765	300	....	
May 3	....	300	....	
May 4	1,490	300	....	
May 5	1,805	300	....	
		Meat (*grams*).		
May 6	1,815	60	....	
May 7	1,825	80	....	
May 8	1,770	180	....	
May 9	1,795	180	....	
May 10	1,805	180	....	
May 11	1,800	180	10.0	No symptoms.
May 12	1,720	180	None.	
May 13	1,815	180	None.	
May 14	1,845	115	10.0	Do.
May 15	1,830	115	10.0	Do.
May 16	1,815	115	10.0	Loss of weight; no other symptoms.
May 17	1,830	115	15.0	No symptoms.
May 18	1,835	115	15.0	Stiffness.
May 19	1,825	115	15.0	No symptoms.
May 20	1,850	115	15.0	A little stiff.
May 21	1,835	115	17.0	No symptoms.
May 22	....	115	17.0	
May 23	1,820	115	17.0	Do.
May 24	1,835	115	20.0	Do.
May 25	1,840	115	20.0	Feces soft and black.
May 26	1,820	115	23.0	
May 27	1,840	115	25.0	A little stiff.
May 28	1,830	115	25.0	
May 29	....	115	None.	
May 30	....	115	None.	
May 31	1,770	115	None.	
June 1	1,765	115	25.0	Diarrhea; stiff in hind legs.
June 2	1,750	115	27.5	Diarrhea and worms.
June 3	1,635	....	27.5	Paralyzed; vomited; died at 3 p. m.

PUP NO. 6.

Date.	Weight.	Food.	Treatment (2 per cent caffein).	Symptoms.
	Grams.	*Milk* (cc).	cc.	
Apr. 28	....	300	....	
Apr. 29	1,280	300	....	
Apr. 30	1,290	300	....	
May 1	1,315	300	....	
May 2	1,330	300	....	
May 3	....	300	....	
May 4	1,360	300	....	
May 5	1,365	300	....	
		Meat (*grams*).		
May 6	1,395	60	....	
May 7	1,365	80	....	
May 8	1,340	180	....	
May 9	1,380	180	....	
May 10	1,400	180	....	
May 11	1,425	180	14.5	No symptoms.
May 12	1,470	180	None.	
May 13	1,485	180	None.	
May 14	1,510	115	14.5	Do.
May 15	1,500	115	14.5	Do.
May 16	1,485	115	14.5	Passed worms.
May 17	1,480	115	14.5	
May 18	1,485	115	19.5	Feces soft and black; almost diarrhea.
May 19	1,495	115	19.5	
May 20	1,500	115	19.5	Scratches her eyes and chases her tail.
May 21	1,500	115	17.0	
May 22	....	115	17.0	
May 23	1,470	115	17.0	
May 24	1,465	115	20.0	
May 25	1,450	115	20.0	Feces soft and black.
May 26	1,450	115	23.0	Diarrhea and worms.
May 27	1,355	115	23.0	Refused to eat all food.
May 28	1,270	115	23.0	Threw up worms, stiff, and has skin over both eyes.
May 29	....	....	....	Found dead.

Highest amount of caffein given, 362 mg per kilo. No autopsy.

Examination of the results obtained in the experiments of series C shows that young and growing dogs tolerate large amounts of caffein. In four subjects of this series, Nos. 1, 2, 3, and 6, no effect was observed when moderately large amounts (160 to 200 mg per kilo of caffein) were fed. Symptoms were noticed only when these amounts of caffein were increased from 50 to 60 per cent. The other two dogs, Nos. 4 and 5, of this series were less resistant, however, to caffein, as 0.16 gram of the drug per kilo induced well-marked symptoms. Since these were fed meat, while Nos. 1, 2, and 3 received milk, the difference in toxicity may be due to the diet employed, but No. 6, which likewise received a meat diet, failed to show the effects of caffein when 200 mg per kilo were fed. On the other hand, it should be noticed that No. 1 died after receiving 360 mg per kilo, No. 2 survived a dose of 334 mg, while No. 3 died after a dose of 322 mg per kilo of caffein. The fatal doses for Nos. 4, 5, and 6 were 287, 335, and 300 mg per kilo, respectively. Although the differences are too small to justify any definite conclusion regarding the effect of a milk diet or of a meat diet on the toxicity of caffein, the results nevertheless suggest a reasonable possibility that caffein is more toxic to young dogs when on an exclusively meat diet than when fed milk. It is perfectly evident, however, that the resistance to caffein in either case is very great, almost twice that of adult subjects. As shown in series A and B, 125 to 175 mg per kilo proved fatal to all but two animals in these experiments, while symptoms of toxicity appeared after much smaller doses. In other respects the behavior of young dogs toward caffein was the same as that of the adult. In neither case was cumulation nor tolerance observed under the conditions of these experiments. The findings at autopsy were likewise similar, as gastro-enteritis was the chief lesion observed on macroscopic examination. It might be mentioned, however, in this connection, that the symptoms of caffein intoxication in young dogs often presented marked differences from those observed in those of more advanced age. The resemblance of the effects of caffein in young puppies and in rabbits was very striking. In both, the tonic with clonic convulsions were observed after a sufficient quantity of caffein was administered. In the dogs which were fully grown a large dose of caffein was usually followed by tonic convulsions and almost instantaneous death.

Moderately large amounts of caffein fed daily to puppies for several days—in some cases as long as 10 days—induced mild symptoms only. No cumulative effect was observed in any of the experiments of series C. There seems to be tolerance of certain functions toward caffein, but no general tolerance of the body could be obtained in these experiments. Caffein is apparently less toxic for adult dogs on high than on low protein diet. In young and growing dogs caffein is somewhat less toxic when milk, rather than meat, forms the exclusive diet. Some pathological conditions apparently increase the toxicity of caffein also in dogs. The symptoms of caffein intoxication observed in young dogs are in some respects different from those in full grown and older animals, and resemble those noticed in rabbits.

DISCUSSION OF RESULTS.

It was pointed out at some length in the introduction that the toxicity of some drugs may not be the same for all forms of life. This observation was also made by some investigators who experimented with caffein on different species of animals. Thus Maurel[56] stated that caffein is twice as toxic for the frog as for the rabbit when administered by mouth. Fröhner's[26] experiments, on the other hand, made on domestic animals, failed to show great differences in the toxicity of caffein. According to this observer, horses seem to be more susceptible than cattle, goats, and swine, the minimum toxic dose being the same for all of these, while the resistance of the dog to caffein is about midway between that of the horse and the other animals mentioned. It may be remarked, however, that Fröhner made only 13 experiments. That these data are inadequate for the formation of any conclusions as to the toxicity of caffein is evident since the most striking effect of caffein observed in the work herein reported was the comparatively wide range of variation in the resistance of individuals of the same species to this drug. This was found to be the case even when the conditions of experimentation were approximately uniform, and was observed whatever the mode of administration of the drug employed. The toxicity for different individuals also varied in acute as well as in chronic intoxication. It is for this reason that the number of tests employed were often quite large, for no conclusions of any value could be drawn without averaging the results of a sufficiently large number of experiments. Furthermore, it is to be borne in mind that the action of a drug may differ according to the mode of its introduction into the body and that different species of animals may vary in this regard. This is especially true of some substances when given by mouth, the range in toxicity for certain species of animals being much greater when thus administered than when injected subcutaneously or intravenously.

Maurel's [56] investigations are of interest in this connection, as his work embraces a systematic study of the toxicity of a large number of substances in the rabbit, pigeon, and frog when given by mouth, subcutaneously, intravenously, or when injected into the muscles. According to this investigator the range of variation of the toxicity of a substance is widest when given by mouth. Potassium sulphocyanid, for example, is about 2.5 times as toxic for the frog as for the rabbit when given by mouth. Quinin hydrobromid is three times as toxic for the frog as for the pigeon, while for the rabbit it is twice as toxic as for the pigeon. When given by hypodermic injection the toxic dose per kilo weight is practically the same for all three species. The difference of resistance according to the mode of administration is even more marked for spartein sulphate. When given by mouth the toxicity for the rabbit is six times as great as for the frog, but when injected subcutaneously the toxic dose is about the same for the rabbit and for the frog. The relation of the mode of administration to toxicity is further shown in the following substances: For the rabbit the minimum fatal dose per kilo of quinin hydrobromid is 1.5 grams administered by mouth, 0.5 gram when injected subcutaneously, and 0.07 gram by the intravenous path, while strychnin sulphate is twice as toxic administered intravenously as subcutaneously, and six times as toxic as when administered by mouth. The mode of introduction, however, does not always affect the toxicity of a substance. This is made evident by the action of strychnin on frogs in which, according to Maurel [56], the toxic dose is the same whether given by mouth or injected into the subcutaneous tissues. This appears to hold true also for other animals as demonstrated by the experiments of Hatcher [35] on the cat, in which he observed that strychnin is as readily absorbed from a full stomach as from the subcutaneous tissues. These findings are extremely interesting, especially in view of Maurel's [57] work on the subject, according to which he finds that a substance is much less toxic when given by mouth than when administered by hypodermic injection or intravenously. That this generalization does not admit, however, of universal application is made evident by the work of various experimenters. Claude Bernard [10] observed that curara is as poisonous for the pigeon when given by mouth as when injected subcutaneously, while Zalesky [86] found that samandarin is more toxic for frogs when introduced into the stomach than by injection into the lymph sacs. Our experiments with caffein likewise show that Maurel's generalization does not always hold good, since it was found in experiments with gray rabbits that the minimum fatal dose is but moderately greater by mouth than by the subcutaneous path.

Equally interesting is the observation of the writer, that in the guinea pig the difference in the toxicity between the subcutaneous and intraperitoneal injections is very slight, while in the cat the toxicity of caffein is the same whether given by mouth or injected into the subcutaneous tissues, and is markedly less when injected into the peritoneal cavity. The experiments on dogs show considerable variation of effective dose when given by mouth, but the interesting observation was made that the toxic dose by mouth may be smaller in some cases than the average dose by subcutaneous injection. If the resistance to caffein by subcutaneous injection of the different species of animals experimented upon in the present research be compared, it will be noticed that the gray rabbit or Belgian hare, which is more resistant than the other varieties employed, stands more caffein in proportion to the weight of the body than the other animals.

Although the minimum fatal dose was found to be somewhat larger for the guinea pig than for the gray rabbit when caffein was injected intraperitoneally, it was on the contrary smaller by other paths of introduction, and approximated quite closely the minimum fatal dose for rabbits of the other varieties. Cats as well as dogs were found to be distinctly less resistant to caffein than the herbivora.

There are a number of factors far more important than zoological differences which influence the toxicity of caffein. Some of these are age, season, and pathologic conditions. As these factors have already been dwelt upon in their appropriate places, further discussion might seem unnecessary, but owing to their importance in determining the action of a drug, emphasis is desirable. Especially is this the case with pathological conditions in relation to toxicity. While no positive proof of diminished resistance to caffein in pathological conditions was obtained by subjecting the suggestion to experimental test, it was observed in these experiments on rabbits that death occurred in some individuals after small doses which are usually not even toxic. The findings at autopsy indicate the presence of pathological conditions. The same was observed in some experiments on cats and dogs. It is extremely probable, therefore, that disease modifies the reaction of the organism to caffein as well as to other drugs.[78]

That the resistance to drugs may vary according to the age of the subject has been maintained by a number of pharmacologists. According to Guinard,[80] young dogs, rabbits, and guinea pigs are very susceptible to morphin, resembling children in this regard.[a] The minimum fatal dose for these animals is about one-third less than for the adult. This is not true, however, for the young of other species. Cats under 15 days of age tolerate twice the toxic dose of morphin for the adult cat. Young beeves and goats are likewise more resistant to this alkaloid than adults. On the other hand, according to Livon,[54] young guinea pigs are more sensitive to alkaloids than adults. The toxicity of caffein, as shown in the present investigation, was found to be less in the young than in the adult. In dogs the young subjects are in some instances almost twice as resistant as adults. The difference was found to be less in cats and rabbits than in dogs, but it was quite marked.

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[a] A case of accidental poisoning reported recently by Wichura (Münich, Med. Woch., 1911, No. 30, p. 1618) throws some doubt on the accepted view that the susceptibility of young children to morphin is greater than that of adults. Wichura also found that the therapeutic doses of codein preparations ordinarily recommended for children in pleuritic cough are not effective in this condition.

The effect of season on the toxicity of drugs has been discussed in the section on the experiments on guinea pigs, which were more resistant to caffein in the fall than in February and March. The effect of season seems to vary with the animal, but it may also differ with the substance employed. In Noe's[65] studies on this subject cantharidin was found to be more toxic for the hedgehog in November than in July. The effect of season was different for morphin, as it was observed that the resistance of the hedgehog was greater at the end of the summer than earlier in the season.

The relation of diet to toxicity of drugs has been studied by Hunt.[39] His experiments indicate that this is an important factor in the resistance to acetonitril. The studies here reported on the effect of diet on toxicity of caffein in rabbits were confined to experiments with oats and carrots and do not show any modification of the resistance to caffein. The question of diet in chronic intoxication in dogs, however, suggests that in these animals diet may affect the toxicity of caffein, although the data on this subject are far from satisfactory. There is nevertheless sufficient evidence to suggest that a high protein diet for the adult dog tends to greater resistance of the animal to caffein and similarly the growing dog tolerates larger quantities of caffein on a milk diet than on a diet of meat.

This brings us to a consideration of the behavior of caffein in chronic intoxication. Although in both rabbits and dogs absence of cumulation was evident, in other respects decided differences in the resistance to caffein were observed. While the rabbit tolerates more than twice the single dose of caffein per kilo for the dog, the result is quite different in repeated dosage of the drug, the rabbit succumbing to continued administration of much smaller doses of the drug than the dog. This is probably due to lesions of the gastro-intestinal canal caused by caffein which occasions loss of appetite much more readily in the rabbit than in the dog. The abundant energy reserve in the dog makes it possible for this animal to stand inanition much longer than the rabbit and other herbivora. The difference in the behavior of the rabbit and dog toward caffein is interesting as showing complete reversal of resistance in acute and chronic intoxication. From the statement in the introduction it is evident that the size of the single toxic or lethal dose of a substance is in no wise an index of the active degree of its toxicity. The experiments with caffein here reported furnish additional evidence that this is true, at least for the rabbit.

GENERAL SUMMARY AND CONCLUSIONS.

The toxicity of caffein in the rabbit varies with the mode of its administration, being least when given by mouth and greatest by intravenous administration. The toxicity is from 15 to 20 per cent greater by subcutaneous injections than by mouth, but is about half of that when injected into the peritoneal cavity. No difference was observed in the toxicity of caffein whether administered into gluteal or into the lumbar muscles. When introduced by this route the toxicity was found to be less by one-third than when it is injected into the peritoneal cavity, but is about 30 per cent more toxic than the subcutaneous injections. White or black rabbits were found to be less resistant to caffein than gray rabbits.

The resistance of the guinea pig to caffein, as of the rabbit, is greatest when given by mouth. The minimum fatal dose is less by intraperitoneal injections, but greater than by subcutaneous injections, thus differing from the rabbit in this regard. The adult cat is less resistant than the guinea pig or rabbit to caffein. The minimum lethal dose by mouth is the same as by subcutaneous, and is less than by intraperitoneal, injection. The minimum fatal dose for dogs was found to be the same by mouth as by subcutaneous injection and is almost the same as for the cat. The toxicity of caffein varies in the guinea pig according to season of the year.

Age is likewise a factor in the toxicity of caffein, young animals being more resistant than the full-grown and older animals; this was shown in experiments on rabbits, cats, and dogs. The symptoms of caffein poisoning also were different in puppies and in full-grown dogs. Different diets, such as carrots and oats, did not influence the resistance of rabbits and guinea pigs to caffein. Low protein diet tends to decrease resistance to caffein in dogs. Young growing dogs are less resistant to caffein on a meat than on a milk diet. Caffein is not cumulative in the rabbit or dog, even if administered for a considerable length of time. Some degree of tolerance may be induced in the rabbit under certain conditions, but not in dogs under the conditions of the experiments made in this investigation. The possibility, however, that dogs may acquire tolerance for caffein is not excluded. Although the rabbit tolerates a much larger single dose of caffein than the dog, it was found, in experiments on chronic intoxication that the rabbit is less resistant to caffein than the dog. The toxicity of caffein is probably increased under pathological conditions, since comparatively smaller doses were fatal to rabbits, cats, and dogs, when marked lesions not due to caffein were found at autopsy. Glycosuria was observed in rabbits, guinea pigs, and cats when caffein was given in sufficient amounts.

TABLE 18.—*Acute caffein intoxication: Table showing average minimum toxic and minimum fatal doses for adult animals.*

Animal.	Effect of dose.	Dose per kilo (grams.)				
		Subcutaneously.	By mouth.	Intraperitoneally.	Intramuscular.	Intravenous.
Rabbit, gray	Toxic	0.15	0.325	0.100-0.125	0.13-0.15	0.05
	Fatal	.30	.350	.150	.20	0.10- .16
Rabbit, white or black	Toxic	....	....	....	....	....
	Fatal	.20	.290	....	....	....
Guinea pig	Toxic	0.15- .16	.150	.200	....	....
	Fatal	.20- .24	0.280- .300	.240- .250	....	....
Cat	Toxic	.12- .14	.125	.125- .150	....	....
	Fatal	.15	.150	.180- .200	....	....
Dog	Toxic	....	.100- .120	....	....	....
	Fatal	.15- .16	.140- .150	....	....	....

NOTE.—The doses given in this table are approximate.

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U. S. DEPARTMENT OF AGRICULTURE,

BUREAU OF CHEMISTRY—BULLETIN No. 149.

H. W. WILEY, Chief of Bureau.

THE GROWTH OF WHEAT SEEDLINGS AS AFFECTED BY ACID OR ALKALINE CONDITIONS.

BY

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LETTER OF TRANSMITTAL.

U. S. DEPARTMENT OF AGRICULTURE,
BUREAU OF CHEMISTRY,
Washington, D. C., June 12, 1911.

SIR: I have the honor to submit for your approval a manuscript prepared by J. F. Breazeale and J. A. Le Clerc, plant physiological chemists, of this bureau, on the growth of wheat seedlings as affected by acid and alkaline conditions. Although the results herein recorded were obtained from experiments conducted in water cultures, it is believed that their interpretation may be directly applied to practical agriculture. I recommend the publication of this manuscript as Bulletin No. 149 of the Bureau of Chemistry.

Respectfully,

H. W. WILEY,
Chief of Bureau.

Hon. JAMES WILSON,
Secretary of Agriculture.

CONTENTS.

	Page.
Introduction	5
Experiments with nutrient solutions and distilled water cultures	6
Description of solutions and method of sowing	6
First crop of seedlings	6
Second crop of seedlings	12
Comparison of the first and second crops	13
Experiments using aluminum and ferric hydroxids to reduce acidity of solution	15
Experiments using clover and timothy with wheat	16
Conclusions	17

ILLUSTRATIONS.

	Page.
PLATE I. Fig. *a*.—First crop of plants, grown 9 days in (1) distilled water; (2) distilled water+calcium carbonate; (3) sodium nitrate; (4) sodium nitrate+calcium carbonate. Fig. *b*.—First crop of plants, grown 9 days in (1) distilled water; (2) distilled water+calcium carbonate; (3) sol. 5, potassium chlorid; (4) sol. 6, potassium chlorid+calcium carbonate; (5) sol. 9, hydrochloric acid; (6) sol. 10, hydrochloric acid+calcium carbonate	8
II. Fig. *a*.—First crop of plants, grown 9 days in (1) distilled water; (2) distilled water+calcium carbonate; (3) sol. 7, potassium sulphate; (4) sol. 8, potassium sulphate+calcium carbonate; (5) sol. 11, sulphuric acid; (6) sol. 12, sulphuric acid+calcium carbonate. Fig. *b*.—First crop of plants, grown 9 days in (1) distilled water; (2) sol. 3, sodium nitrate; (3) sol. 7, potassium sulphate; (4) sol. 13, sodium nitrate+potassium sulphate	8
III. Fig. *a*.—Second crop of plants, grown 5 days in residual solutions (1) distilled water; (2) distilled water+calcium carbonate; (3) sodium nitrate; (4) sodium nitrate+calcium carbonate. Fig. *b*.—Second crop grown 5 days in residual solutions (1) distilled water; (2) distilled water+calcium carbonate; (3) sol. 5, potassium chlorid; (4) sol. 6, potassium chlorid+calcium carbonate; (5) sol. 9, hydrochloric acid; (6) sol. 10, hydrochloric acid+calcium carbonate	12

Page.

IV. Fig. *a.*—Second crop of plants, grown 5 days in residual solutions (1) distilled water; (2) distilled water+calcium carbonate; (3) sol. 7, potassium sulphate; (4) sol. 8, potassium sulphate+calcium carbonate; (5) sol. 11, sulphuric acid; (6) sol. 12, sulphuric acid+calcium carbonate. Fig. *b.*—Second crop of plants, grown 5 days in residual solutions (1) distilled water; (2) sol. 3, sodium nitrate; (3) sol. 7, potassium sulphate; (4) sol. 13, sodium nitrate and potassium sulphate 12

V. Fig. *a.*—Crop grown 5 days in (1) distilled water; (2) distilled water+aluminum hydrate; (3) potassium chlorid; (4) potassium chlorid+aluminum hydrate; (5) hydrochloric acid; (6) hydrochloric acid+aluminum hydrate. Fig. *b.*—Crop grown 5 days in (1) distilled water; (2) distilled water+aluminum hydrate; (3) potassium sulphate; (4) potassium sulphate+aluminum hydrate; (5) sulphuric acid........ 16

VI. Fig. *a.*—Crop grown 5 days in (1) distilled water; (2) distilled water+aluminum hydrate; (3) sodium nitrate; (4) sodium nitrate+aluminum hydrate; (5) sodium hydrate; (6) sodium hydrate+aluminum hydrate. Fig. *b.*—Crop grown 5 days in (1) distilled water; (2) sodium nitrate; (3) potassium chlorid; (4) potassium sulphate; (5) potassium nitrate........ 16

VII. Fig. *a.*—Crop grown 5 days in (1) distilled water; (2) distilled water+ferric hydrate; (3) potassium chlorid; (4) potassium chlorid+ferric hydrate; (5) hydrochloric acid; (6) hydrochloric acid+ferric hydrate. Fig. *b.*—Crop grown 5 days in (1) distilled water; (2) distilled water+ferric hydrate; (3) potassium sulphate; (4) potassium sulphate+ferric hydrate; (5) sulphuric acid; (6) sulphuric acid+ferric hydrate........ 16

VIII. Fig. *a.*—Comparison of entire pan of wheat plants grown in (1) distilled water; (2) potassium sulphate; and (3) potassium sulphate+calcium carbonate. Fig. *b.*—Clover plants grown with wheat in (1) distilled water; (2) distilled water+calcium carbonate; (3) potassium sulphate; (4) potassium sulphate+calcium carbonate........ 16

THE GROWTH OF WHEAT SEEDLINGS AS AFFECTED BY ACID OR ALKALINE CONDITIONS.

INTRODUCTION.

The following physiological study on the effect of the reaction of the culture medium on the growth of wheat seedlings and particularly on the development of the root was undertaken primarily to determine whether or not the results obtained in practical agriculture can be explained in the laboratory from a purely scientific standpoint.

A few years ago Director Thorne, of the Ohio experiment station, called the attention of one of the authors to peculiar conditions existing on certain of his fertilizer experiment plots, to which potassium chlorid and potassium sulphate had been applied annually for the past 12 years. These plots had become so acid that it was found impossible to grow clover on them. Liming one-half of each plot had restored that half to almost its original fertility. This acidity, he said, was due to the selective action of the root, doubtless a correct explanation which will be considered in detail later.

There seems to be a natural tendency for all soils to become acid under continuous culture, due chiefly to one of two processes. The primary cause of this acidity is the decay of organic matter, leaves, stems, etc., which during decomposition develop acid-reacting bodies. If this process is allowed to continue, the reaction will ultimately become alkaline, but under soil conditions, especially if the aeration is poor and the water level high, decay is apt to be checked, leaving the soil in a characteristic acid condition. This phase of soil acidity, which will not be considered in this bulletin, has been studied extensively during the past few years, particularly by the Rhode Island and Ohio experiment stations, and by Mr. Frederick V. Coville[1] in his elaborate investigations on the blueberry. Peat bogs furnish an exaggerated case of acidity, due to organic matter. The secondary cause of soil acidity is the subject of the investigation herein reported.

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[1] U. S. Dept. Agr., Bureau of Plant Industry Bul. 193.

EXPERIMENTS WITH NUTRIENT SOLUTIONS AND DISTILLED WATER CULTURES.

DESCRIPTION OF SOLUTIONS AND METHOD OF SOWING.

The following experiments were carried on in nutrient solutions and in distilled water cultures, eliminating the disturbing factors due to the presence of soil grains. For the most part wheat was used on account of the ease in handling the young seedlings. The seed, which was furnished by the Pennsylvania experiment station, was washed with warm water for about six hours and then sprinkled on perforated aluminum disks about 12 inches in diameter. These disks were floated, by means of glass tubes, in shallow pans which held about 3 liters of water. In this way approximately 1,000 seeds were used in each pan. The preliminary washing removed all of the readily soluble salts, the actual amounts so removed, as has been shown by the authors in previous experiments[1] of this kind, being quite large. The distilled water was prepared especially for this purpose and a small amount of carbon black was placed in each pan.

After some preliminary work a set of 13 pans was started, as follows:

Solution 1. Distilled water.
Solution 2. Distilled water + 0.5 gram of calcium carbonate.
Solution 3. 150 parts per million of sodium nitrate.
Solution 4. 150 parts per million of sodium nitrate + 0.5 gram of calcium carbonate.
Solution 5. 150 parts per million of potassium chlorid.
Solution 6. 150 parts per million of potassium chlorid + 0.5 gram of calcium carbonate.
Solution 7. 150 parts per million of potassium sulphate.
Solution 8. 150 parts per million of potassium sulphate + 0.5 gram of calcium carbonate.
Solution 9. 10 parts per million of hydrochloric acid.
Solution 10. 10 parts per million of hydrochloric acid + 0.5 gram of calcium carbonate.
Solution 11. 10 parts per million of sulphuric acid.
Solution 12. 10 parts per million of sulphuric acid + 0.5 gram of calcium carbonate.
Solution 13. 150 parts per million of sodium nitrate + 75 parts per million of potassium sulphate.

FIRST CROP OF SEEDLINGS.

These cultures were started January 24 and grown until February 2. The plants were then harvested, representative samples were selected and photographed, and the others were dried, weighed, and prepared for analysis. The photographs are shown in Plates I and II, the controls being the same in each case. The solutions were then made up to volume, 500 cc withdrawn for analysis, and the remainder saved in which to grow a second crop.

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[1] U. S. Dept. Agr., Bureau of Chemistry Bul. 138.

Reaction of the residual solution.—In Table 1 are shown the reactions and titration data for the 13 solutions after gathering the first crop; no nitrates and only a trace of potash remained in any of the solutions.

TABLE 1.—*Titration of the culture solutions after the first crop was harvested.*

No. of solution.	Original solution.[1]	Reaction upon boiling.	Titration of 100 cc with twentieth-normal sodium hydrate.
			cc.
1	Distilled water	Slightly acid	0.1
2	Distilled water+calcium carbonate	Akaline	
3	Sodium nitrate	do.	
4	Sodium nitrate+calcium carbonate	do.	
5	Potassium chlorid	Acid	.8
6	Potassium chlorid+calcium carbonate	Alkaline	
7	Potassium sulphate	Acid	1.2
8	Potassium sulphate+calcium carbonate	Alkaline	
9	Hydrochloric acid	Acid	.1
10	Hydrochloric acid+calcium carbonate	Alkaline	
11	Sulphuric acid	Acid	.2
12	Sulphuric acid+calcium carbonate	Alkaline	
13	Sodium nitrate+potassium sulphate	do	

[1] See page 6 for amounts of constituents in each solution.

Effects of sodium nitrate.—In Plate I, figure *a*, a slight improvement, especially in root development, is noticed in the distilled-water cultures when calcium carbonate is added, but no appreciable difference is seen when the calcium carbonate is added to the sodium nitrate solution. By reference to the analysis of the culture solutions in Table 1, it will be seen that control No. 1, at the close of the experiment, showed, upon boiling, a slight acid reaction. This was probably due either to the exudation by the roots of some acid-reacting body or to the excretion of similar bodies by the seeds themselves. Nos. 2 and 4 were, of course, alkaline because of the presence of the calcium carbonate. The alkalinity of No. 3 was due to the fact that all of the nitrate (NO_3) or acid radical in the sodium nitrate had been removed by the plant, while some of the sodium or alkaline radical, being less rapidly absorbed, remained in the solution, forming, probably, sodium hydroxid. This, in turn, had taken up carbon dioxid and was existing in the solution as sodium bicarbonate.

Relative effects of potassium chlorid and hydrochloric acid.—In Plate I, figure *b*, are shown the relative effects of potassium chlorid and hydrochloric acid (solutions 5 and 6, and 9 and 10). The plants removed practically all of the potash from the potassium chlorid solution, only a trace remaining, while by analysis 38 parts per million of chlorin out of a total of 71 still remained in the solution, thus clearly showing the selective ability which roots possess to take up one part or radical of a salt and to leave the other. All of this chlorin did not exist as hydrochloric acid, for after boiling and titrating with twentieth-normal sodium hydrate, using phenolphthalein as an indicator, a reading equivalent to only 14.6 parts per million of hydrochloric acid was obtained, which, however, is a much larger amount of free acid than was originally present in solutions 9 and 10, where only 10 parts per million of acid were used.

The effects of the strongly acid solutions were markedly shown upon the roots, that is, in the potassium-chlorid solution they showed the same general characteristics as those grown in the hydrochloric acid; they were short, stubby, and often discolored, the tips being enlarged, and many of the roots growing in the shape of a fishhook, a characteristic of roots grown in the presence of some toxic substance. Moore, Roaf, and Knowles,[1] as a result of their investigation, found that the smallest amount of free acid arrests the growth and nuclear divisions at the root tip, causing it to become thicker, while dilute alkali stimulates the tip to excessive nuclear division.

Of the 10 parts per million of hydrochloric acid added to solution 9, only 1.8 parts remained in solution after the first cropping. The remainder was either absorbed by the plants or the carbon black, or was neutralized by the aluminum disk. Both in the case of the potassium chlorid and of the hydrochloric acid, the presence of calcium carbonate overcame the injurious effects of the acids in the solutions. That is, of course, a common observation and hardly requires citations from the literature to substantiate it. Claudel and Crochetelle[2] found that when they used ammonium sulphate, potassium chlorid, or potassium sulphate in amounts varying from 500 to 5,000 parts per million, an injurious action on germination was noted, and that the application of lime neutralized this toxic effect.

The results obtained by the use of lime in the experiments here reported can be explained only by assuming that the lime has been used to neutralize the acids.

Effect of reaction of the solution on weight of roots and tops.—In making the culture medium alkaline, the growth of the root has been most favorably affected. From Table 2 it is seen that in every case where lime was present the weight of the roots was greater than in the corresponding case without lime, and especially was this so where hydrochloric acid and sulphuric acid had been used, with and without lime.

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[1] Effects of Variations in Inorganic Salts and the Reactivity of the External Medium upon the Nutrition, Growth, and Cell Division in Plants and Animals. Biochem. J., 1908, *3:* 279.

[2] Les engrais et la germination. Ann. Agron., 1896, *22:* 131

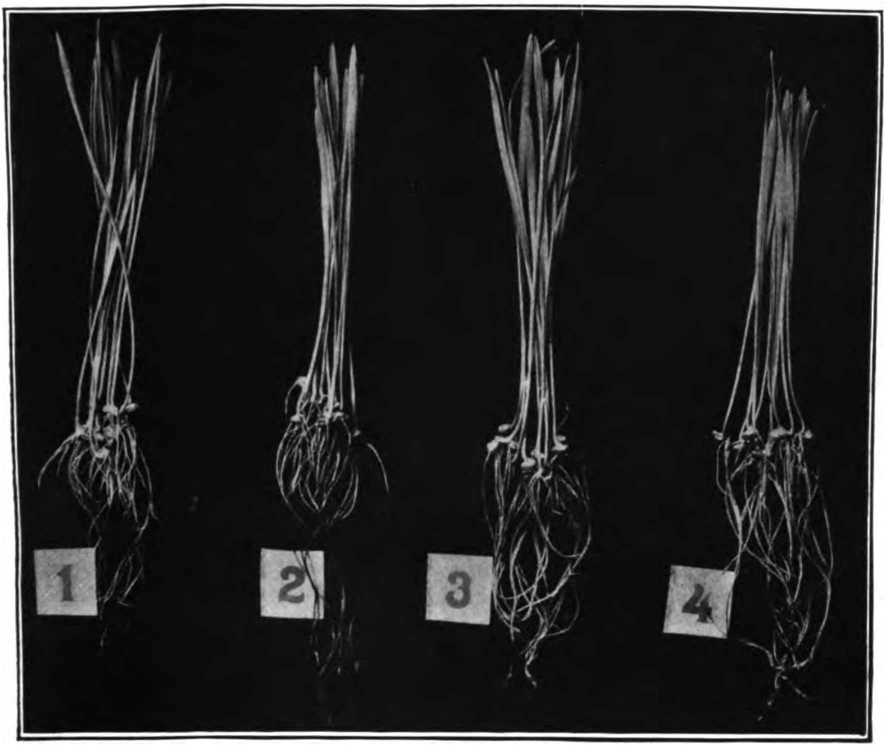

Fig. a.—First crop of plants, grown 9 days in (1) distilled water; (2) distilled water + calcium carbonate; (3) sodium nitrate; (4) sodium nitrate + calcium carbonate.

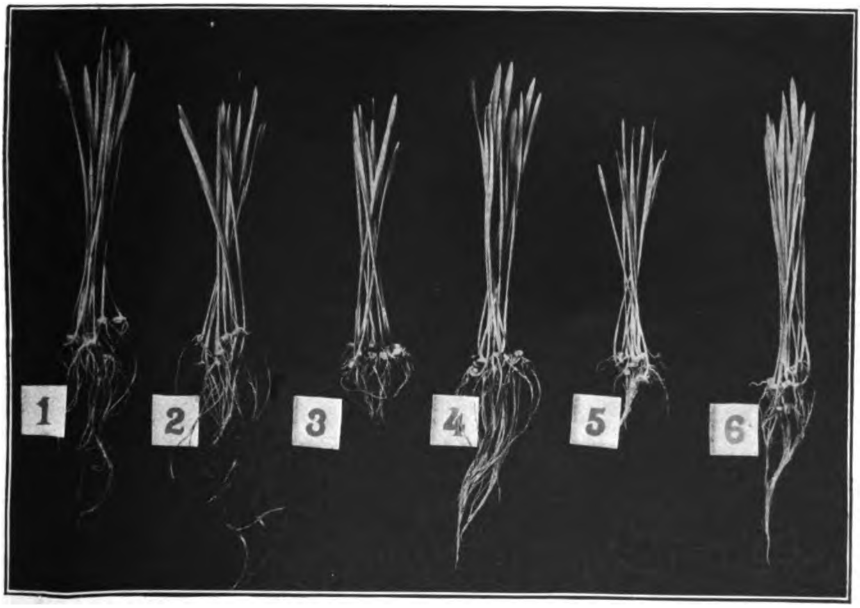

Fig. b.—First crop of plants, grown 9 days in (1) distilled water; (2) distilled water + calcium carbonate; (3) sol. 5, potassium chlorid; (4) sol. 6, potassium chlorid + calcium carbonate; (5) sol. 9, hydrochloric acid; (6) sol. 10, hydrochloric acid + calcium carbonate.

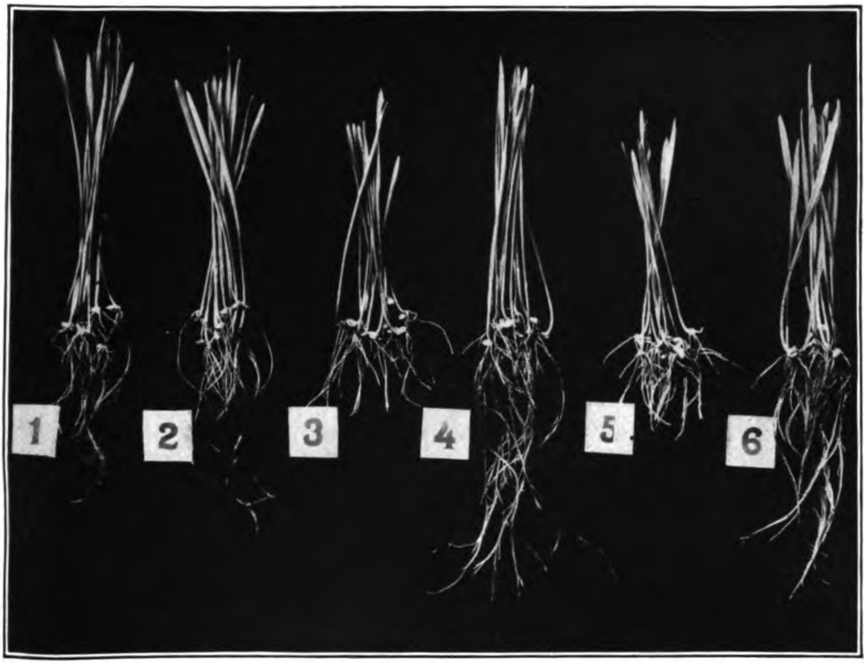

Fig. a.—First crop of plants, grown 9 days in (1) distilled water; (2) distilled water + calcium carbonate; (3) sol. 7, potassium sulphate; (4) sol. 8, potassium sulphate + calcium carbonate; (5) sol. 11, sulphuric acid; (6) sol. 12, sulphuric acid + calcium carbonate.

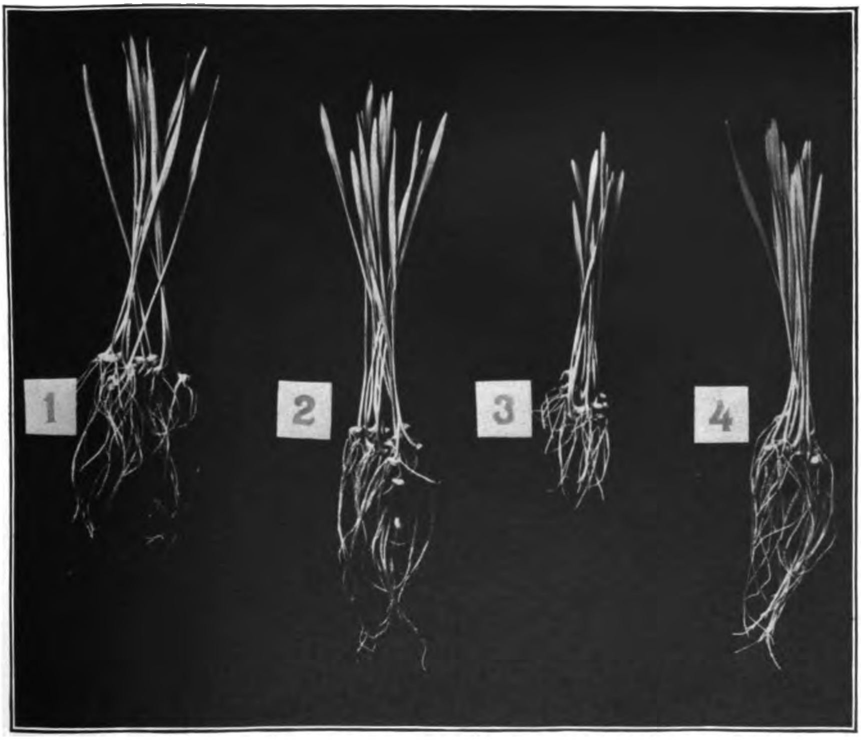

Fig. b.—First crop of plants, grown 9 days in (1) distilled water; (2) sol. 3, sodium nitrate; (3) sol. 7, potassium sulphate; (4) sol. 13, sodium nitrate + potassium sulphate.

TABLE 2.—*Dry weight of first crop of plants.*

No. of solution.	Original solution.	100 tops.	100 roots.	100 whole plants.
		Grams.	*Grams.*	*Grams.*
1	Distilled water	1.013	0.575	2.041
2	Distilled water+calcium carbonate	.953	.704	2.176
3	Sodium nitrate	1.012	.625	2.090
4	Sodium nitrate+calcium carbonate	.932	.673	2.135
5	Potassium chlorid	1.088	.466	2.213
6	Potassium chlorid+calcium carbonate	1.013	.639	2.247
7	Potassium sulphate	1.055	.457	2.342
8	Potassium sulphate+calcium carbonate	.933	.672	2.288
9	Hydrochloric acid	.870	.320	2.103
10	Hydrochloric acid+calcium carbonate	.888	.577	2.185
11	Sulphuric acid	.914	.392	1.910
12	Sulphuric acid+calcium carbonate	1.063	.682	2.092
13	Sodium nitrate+potassium sulphate	.878	.528	2.055

The following tabulation shows the increase in weight of one hundred roots in each case when lime was added:

Solution.	Gram.	Solution.	Gram.
Distilled water	0.129	Potassium sulphate	0.215
Sodium nitrate	.048	Hydrochloric acid	.257
Potassium chlorid	.173	Sulphuric acid	.290

These figures indicate that the least increase was found in the sodium nitrate solution. This is explained by Table 1, which shows that even when sodium nitrate was used alone the solution was alkaline at the end of the experiment, and therefore the further use of calcium carbonate simply acted as a stimulant to a very slight extent.

The weight of the tops, or rather of the stems grown in solutions with and without lime, show no such marked difference, indicating that the appearance of the stems of young wheat seedlings is not always a criterion of the vigor of the plant and therefore of the probable yield of the mature crop. In other words, the root system may be a better indicator as to the probable success of a crop than the appearance of the portion above ground.

The effects on the root development, due to the acidity, are always easily noticeable in water-culture experiments such as these, but in field tests the results of applications of single fertilizers, for example, potassium chlorid, potassium sulphate, ammonium chlorid, or ammonium sulphate, are not necessarily apparent in so short a time, due to the fact that there may be many substances in soils which neutralize the acid thus formed.

Relative effects of potassium sulphate and sulphuric acid.—In Plate II, figure *a*, are shown in a similar way the comparative effects of potassium sulphate and sulphuric acid. With the potassium sulphate the plants removed all of the potash, but left 57 out of a total of 82 parts per million of sulphate in solution. This solution showed on analysis 29.4 parts per million of sulphuric acid, an amount more than twice as large as that originally present in solution 11, thus again showing the extent to which the selective action of roots for one radical of a salt may be carried. Leclerc du Sablon[1] put forth the theory that such useful ions as potassium (K), phosphorus (PO_4), nitrogen (NO_3), and iodin (I) combine with the organic substance of the plant, thus relieving the osmotic pressure within the plant and permitting a still larger quantity of these ions to be absorbed. On the other hand, such ions as chlorin (Cl) and sodium (Na) do not form this organic combination, the result being that the pressure inside the plant soon equals that outside, and the plant is, therefore, satisfied with a relatively small amount of them.

Of the 10 parts per million of sulphuric acid present in solution 11, all but 4.9 parts per million disappeared. The appearance of the plant roots in the potassium sulphate solution was even worse than that of those grown in potassium chlorid. Maze[2] obtained results which may be similarly interpreted in his experiments in growing corn in water cultures. He found that when ammonium sulphate was used the crop was not so well developed as when ammonium chlorid was applied. Calcium carbonate overcame the injurious effects in these as in the preceding cultures, thus again showing how beneficial it is to the crop to maintain the alkalinity of the culture medium.

The effect of sodium nitrate and potassium sulphate separately and in combination.—In Plate II, figure *b*, are shown the cultures of sodium nitrate (alkaline reacting), potassium sulphate (acid reacting), and a mixture of the two. The mixture was prepared so as to become alkaline and produce good plants, and it did so. In this case the nitrate of the sodium nitrate and the potassium of the potassium sulphate were drawn up into the plant, while the sodium left in solution from the sodium nitrate combined with the sulphate from the potassium sulphate to form sodium sulphate, with a sufficient excess of sodium to make the solution alkaline.

Discussion of data obtained on the first crop.—All of the culture solutions to which no sulphate was added; that is, the sodium nitrate, the hydrochloric acid, and the distilled water cultures, contained a small amount of SO_4, due probably to its excretion from the seed. In solutions 7 and 8, however, containing 150 parts per million of potassium sulphate, the amounts of SO_4 were noticeably large, 57 out of the 87 parts per million being found in the solution. This was about 6 times more than was found in the sulphate-free solutions and would seem to indicate that the plant at this stage does not require much more than 25 to 30 parts per million of SO_4, since a much larger amount was at their disposition during the whole period without any larger amount being absorbed. These two solutions, Nos. 7 and 8, containing 87 parts per million of SO_4 and 63 parts per million of potash, respectively, show very clearly to what extent selective action is practiced by the roots. At the end of the experiment all of the potash had been taken up by the plant, while only 30 parts per million of SO_4 were thus absorbed.

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[1] Dégagement d'eau par les plantes, Rev. gén. Bot., 1909, *21:* 295.

[2] Compt. rend., 1911, *152:* 783.

Solutions 5 and 6 show the same selective process going on in the case of potassium chlorid. These solutions contained at the beginning of the experiment 79 parts per million of the potash ion and 71 parts per million of the chlorid ion. At the end of the experiment no potash was found in the solution, indicating that the plant had absorbed the whole of this element, while 35 to 38 parts per million of chlorid were still present. These results are at variance with those obtained by J. de Rufz de Lavison,[1] who, after growing the young seedling five days in twentieth-normal potassium-chlorid solution, found that the concentration of the solution was about equal to that originally used. The probable reason for such a deduction is that a potassium-chlorid solution of twentieth-normal strength is equivalent to 3,700 parts per million, a solution so strong that even if the plant did exercise a normal selective action of 30 to 50 parts per million, it would be most difficult to detect such a change in a solution of the concentration mentioned.

The pans containing (1) the control, (2) the potassium-sulphate solution, and (3) the potassium sulphate with calcium carbonate, as given in Plate VIII, figure *a*, show that the differences in the development of the roots are marked and that the few plants selected for the plates previously shown were not exceptions to the rule. In every case the plants grown in the presence of lime presented a healthier and more vigorous appearance than those grown in the corresponding solutions without lime.

While the tops of the plants grown in the culture solutions containing calcium carbonate were larger and presented a much better appearance than the others, yet their dry weight was not necessarily greater; on the other hand, the dry weight of the roots was increased in every case by the addition of calcium carbonate.

Although the original seeds and the seedlings all contained sulphur in organic combination, yet no weighable amount of inorganic sulphate was found in the ash of the plants, except in those to which sulphate or sulphuric acid had been added.

In the solutions to which calcium carbonate had been added the carbon dioxid which was given off by the roots combined with the calcium carbonate and brought a relatively high amount of lime into solution as calcium bicarbonate, making the solutions distinctly alkaline.

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[1] Du rôle électif de la racine dans l'absorption des sels. Comp. rend., 1910, *151:* 675.

SECOND CROP OF SEEDLINGS.

Reaction of residual solution and weight of crop.—After the first crop of seedlings was removed from the pans, more seeds that had been thoroughly washed were sprinkled on the aluminum disks and allowed to germinate and grow as a second crop for five days. Samples were then photographed, as shown in Plates III and IV. The solutions were again made up to their original volumes and titrated with the results given in Table 3.

TABLE 3.—*Titration of culture solutions after harvesting the second crop of seedlings.*

No. of solution.	Original treatment.[1]	Reaction upon boiling.	Titration of 100 cc with twentieth-normal sodium hydrate.
			cc.
1	Distilled water	Acid	0.3
2	Distilled water+calcium carbonate	Alkaline	
3	Sodium nitrate	Acid	.7
4	Sodium nitrate+calcium carbonate	Alkaline	
5	Potassium chlorid	Acid	3.3
6	Potassium chlorid+calcium carbonate	Alkaline	
7	Potassium sulphate	Acid	1.5
8	Potassium sulphate+calcium carbonate	Alkaline	
9	Hydrochloric acid	Acid	.5
10	Hydrochloric acid+calcium carbonate	Alkaline	
11	Sulphuric acid	Acid	.6
12	Sulphuric acid+calcium carbonate	Alkaline	
13	Sodium nitrate+potassium sulphate	Acid	.5

[1] For full description of solutions, see p. 6.

The plants were dried and weighed as in the case of the first crop, the results being shown in Table 4.

TABLE 4.—*Weight of second crop of plants.*

No. of solution.	Original treatment.	Green weight of 100 tops.	Dry weight.		
			100 tops.	100 roots.	100 residual seeds.
		Grams.	*Grams.*	*Gram.*	*Gram.*
1	Distilled water	7.04	1.51	0.499	0.500
2	Distilled water+calcium carbonate	9.25	1.86	.770	.298
3	Sodium nitrate	9.00	1.80	.564	.398
4	Sodium nitrate+calcium carbonate	10.62	1.98	.728	.270
5	Potassium chlorid	6,14	1.49	.434	.616
6	Potassium chlorid+calcium carbonate	9.78	1.89	.700	.390
7	Potassium sulphate	5.62	1.40	.446	.772
8	Potassium sulphate+calcium carbonate	9.22	1.94	.698	.332
9	Hydrochloric acid		1.21	.338	.378
10	Hydrochloric acid+calcium carbonate	10.54	2.09	.734	.380
11	Sulphuric acid	7.08	1.50	.518	.362
12	Sulphuric acid+calcium carbonate	9.64	1.62	.652	.314
13	Sodium nitrate+potassium sulphate	9.12	1.76	.574	.376

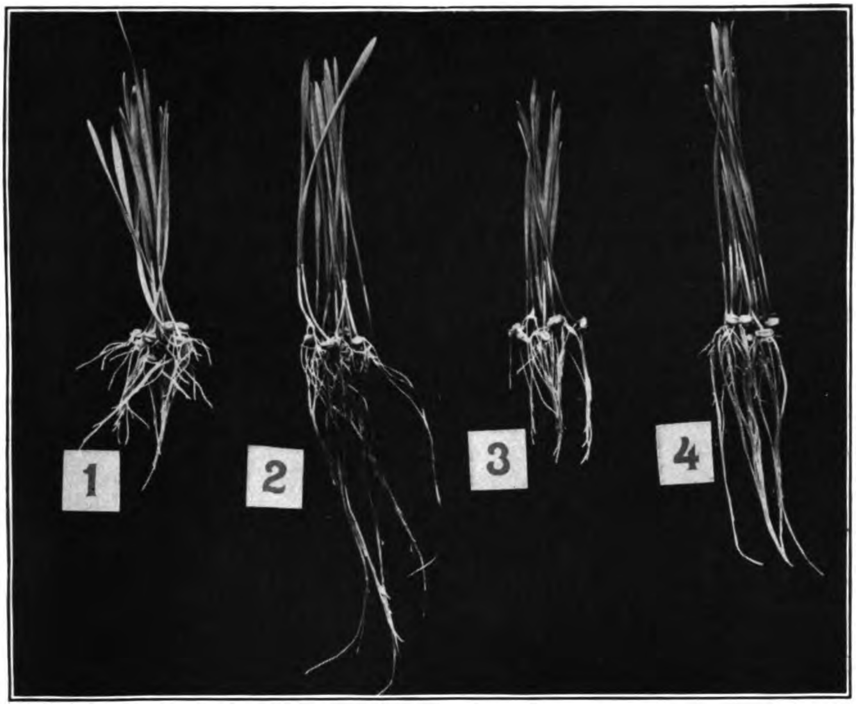

Fig. a.—Second crop of plants, grown 5 days in residual solutions (1) distilled water; (2) distilled water + calcium carbonate; (3) sodium nitrate; (4) sodium nitrate + calcium carbonate.

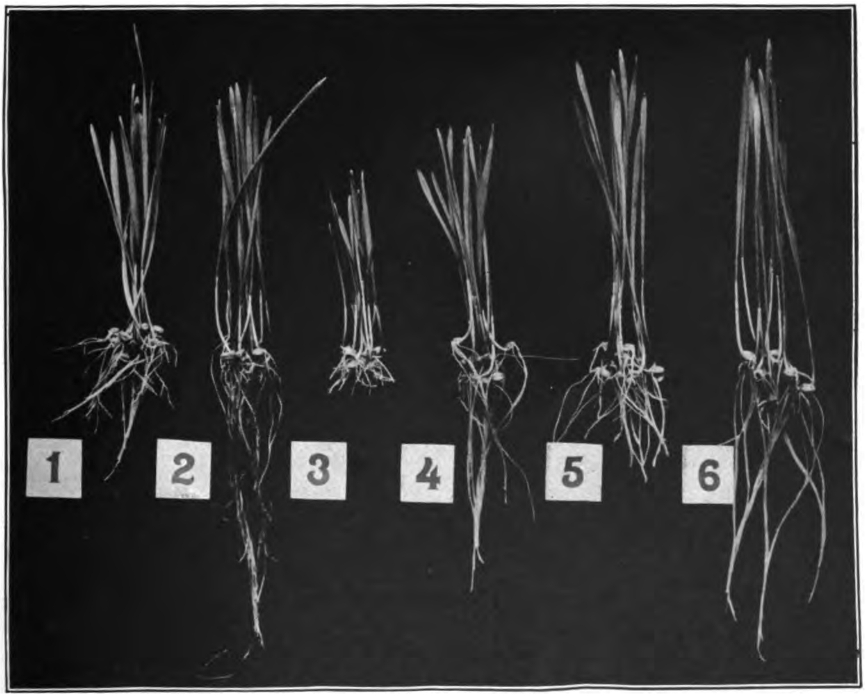

Fig. b.—Second crop, grown 5 days in residual solutions (1) distilled water; (2) distilled water + calcium carbonate; (3) sol. 5, potassium chlorid; (4) sol. 6, potassium chlorid + calcium carbonate; (5) sol. 9, hydrochloric acid; (6) sol. 10, hydrochloric acid + calcium carbonate.

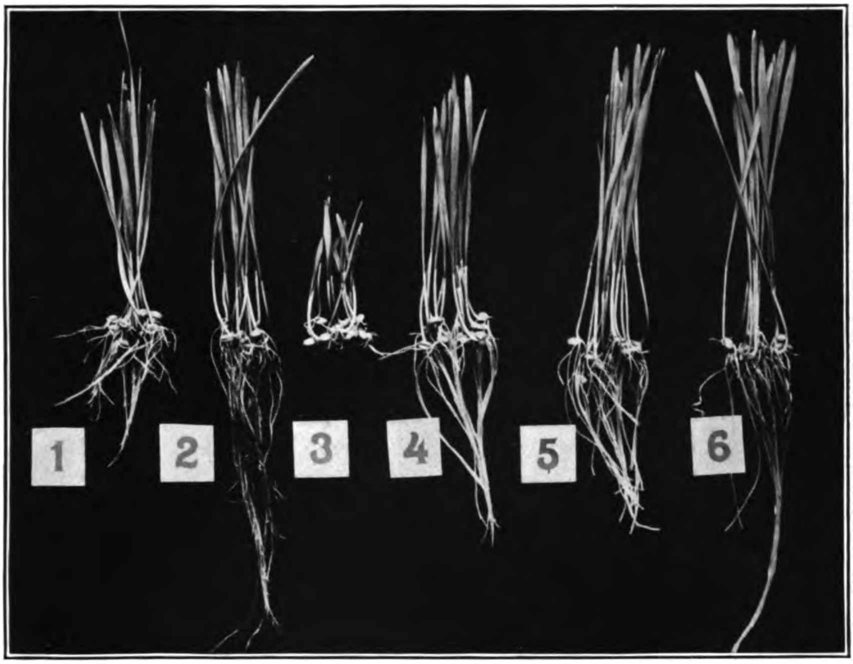

Fig. a.—Second crop of plants, grown 5 days in residual solutions (1) distilled water; (2) distilled water + calcium carbonate; (3) sol. 7, potassium sulphate; (4) sol. 8, potassium sulphate + calcium carbonate; (5) sol. 11, sulphuric acid; (6) sol. 12, sulphuric acid + calcium carbonite.

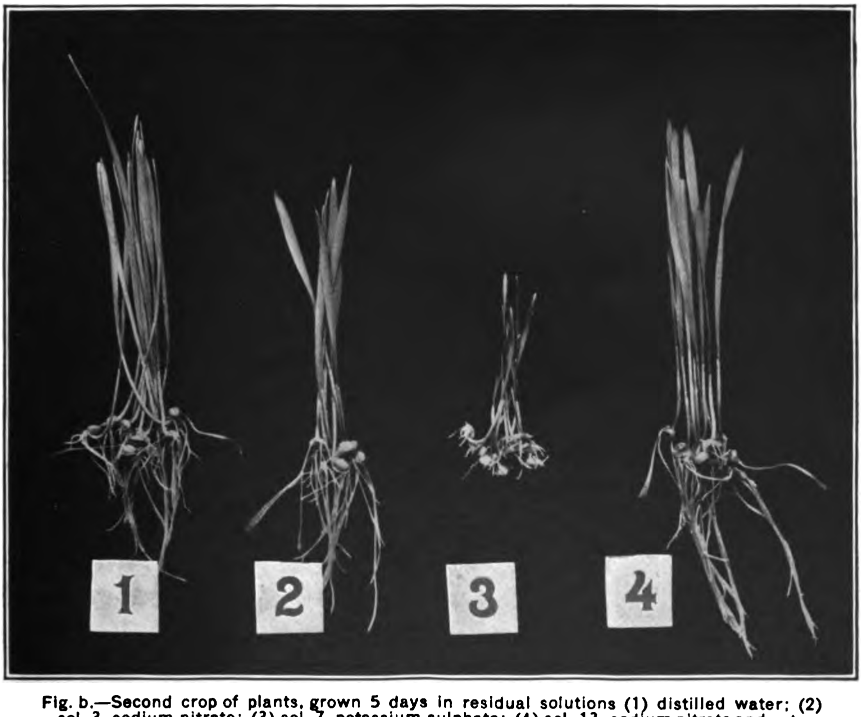

Fig. b.—Second crop of plants, grown 5 days in residual solutions (1) distilled water; (2) sol. 3, sodium nitrate; (3) sol. 7, potassium sulphate; (4) sol. 13, sodium nitrate and potassium sulphate.

Effects of sodium nitrate.—By reference to Plate III, a marked difference will be noted between the distilled water control and the distilled water with calcium carbonate, a difference much greater than was shown in growing the first crop (see Plates I and II). It will also be noticed from Table 3 that the acidity of the solution has increased threefold since the first cropping. Solution 3, Table 3, containing sodium nitrate, which was alkaline after the first cropping, has now also become acid. The sodium evidently either was absorbed by the plant or was neutralized by some organic acid. Solutions 2 and 4, of course, remained alkaline.

Relative effects of potassium chlorid and hydrochloric acid.—In Plate III, figure *b*, the relative effects of potassium chlorid and hydrochloric acid are again shown. The plants grown in the potassium chlorid solution, which, when the second crop was sown, contained no potash but a considerable amount of acid, made practically no root growth and a very poor leaf development. On the other hand, the plants grown in the hydrochloric-acid solution which contained only one-eighth as much acid made a much better growth both in roots and tops.

Relative effects of potassium sulphate, sodium nitrate, and sulphuric acid.—In Plate IV, figure *a*, are shown the residual effects of the potassium sulphate and sulphuric acid. What has just been said of the potassium-chlorid plants and the hydrochloric-acid plants is in the main true of the potassium-sulphate plants and the sulphuric-acid plants also. Figure *b* illustrates the residual effect of sodium nitrate and potassium sulphate used singly and in combination.

COMPARISON OF THE FIRST AND SECOND CROPS.

A striking difference will be noticed between the plants grown in the potash solutions in the first and second crops, when no calcium carbonate was present. The plants in the first crop had practically as much acidity to contend with during the latter days of their growth as the plants of the second crop had during the first few days of their growth, yet the first crop was much better than the second crop. This difference was not due to the fact that the second crop was deficient in potash, for the control pans containing calcium carbonate but no potash gave excellent plants. It is to be noted that the first crop had to contend only with a gradually increasing amount of acidity due to the absorption of potash from the solution in which it was growing, and it was therefore able to accommodate itself and to endure much more acidity than if it had been placed in the stronger acid solution at first, as was done in the case of the second crop. It has been shown by Mr. Jensen, of the Bureau of Plant Industry, in a piece of unpublished work, that the enzymic activity of a wheat seedling is greatest during the first few days of its growth, this being especially true of the oxydases and peroxydases of the root tip. These enzyms are particularly sensitive to acids, and their destruction would mean a cessation of growth. The first crop had evidently passed the critical period with reference to this enzymic activity before the acidity of the solution had developed to such a degree as to prohibit it. When the second crop was placed in the solution the acidity was high enough to practically prohibit the action of the enzyms and the plants were unable to tide over this critical period. The effect of toxic bodies upon the oxydases and peroxydases, that are so active during the first few days of a plant's growth, is an important factor both in agricultural practice and in scientific research and one that is often overlooked.

Many substances that are classified as plant stimulants or depressants no doubt derive their reputation in a great measure from their effect upon the enzyms. Their beneficial or injurious effects would only be noticed during the first few days of the plant's growth when its enzymic activity is so pronounced.

It will be noticed in the case of the second crop that the alkalinity of the solution increased both the green and the dry weight of the tops and the dry weight of the roots. In the case of the residual seeds that were removed from the plants the reverse is true. The presence of an acid in the solution prevented the food material that was stored up in the seed from being transported into the plant. The residual seeds were therefore much lighter when calcium carbonate was present than when it was absent.

After the second crop had been grown in the solutions described for five days the plants were harvested, separated into tops, roots, and residual seeds, and each part dried and weighed separately. The respective solutions were likewise all tested for acidity and the presence of nitrates and potash. Tables 3 and 4 (see page 12) show the results obtained, and a comparison with Tables 1 and 2 (pp. 7 and 9), respectively, will bring out some interesting changes in the residual solution. After the first cropping solution 1 was slightly acid, but after harvesting the second crop it was three times as acid. Solutions 3 and 13 after the first cropping were alkaline, but after the second one both solutions had become quite acid. Analysis of solution 3 showed that while at the beginning there were 40.5 parts per million of sodium (Na), after gathering the second crop only 8 parts per million remained in solution. The difference shows the probable amount of sodium absorbed by the two crops. Not only was the amount thus reduced from 40.5 to 8 parts per million, but the presence of this small amount of sodium seemed to stimulate an acid excretion on the part of the plants to such an extent that the alkaline solution was not only neutralized but was made even more acid than the distilled water culture. On testing for chlorin, after the second cropping, it was shown that only mere traces of that element were found in any of the solutions except Nos. 5 and 6, in which potassium chlorid was used. Even in solutions 9 and 10, to which 10 parts per million of hydrochloric acid were originally added, only traces of chlorin were found. The amounts of chlorin found in solutions 5 and 6 after the second cropping were approximately 5 parts per million on an average, or about one-seventh as much as was present after the first crop was grown. In a similar test for sulphuric acid in the various solutions it was shown that they all contained appreciable amounts, but that solutions 7, 8, and 13, which had been treated with potassium sulphate, contained about two to three times as much sulphuric acid as the others. This acid was apparently either not absorbed to the extent that the chlorin was or else was excreted by the roots in a larger quantity.

The weights of the roots in both crops and of the stems in the second one, when grown with lime, were much greater than when grown without it. The weight of the residual seed, on the other hand, was greater in the unlimed solutions, thus indicating that in an alkaline medium the process of translocation of the seed constituents goes on more readily and completely than under the influence of an acid reaction.

EXPERIMENTS USING ALUMINUM AND FERRIC HYDROXIDS TO REDUCE ACIDITY OF SOLUTION.

A second series of experiments was made, using aluminum hydrate instead of calcium carbonate to keep the solutions from becoming acid. This aluminum hydrate was prepared from aluminum sulphate by precipitation with ammonium hydrate and was washed free from impurities. It was not allowed to dry, but was added as a thick cream. Carbon black was kept in all of the cultures as in the calcium carbonate series. Plate V shows the effect of the aluminum hydrate used instead of lime in the solutions containing potassium chlorid, hydrochloric acid, potassium sulphate, and sulphuric acid. The results after the plant had grown five days indicate that many substances, which are capable of neutralizing the acid of the culture medium and of keeping it alkaline, may be of benefit to the seedling.

In Plate VI, figure *a*, is shown the relative effect of the addition of aluminum hydrate to solutions containing 150 parts per million of sodium nitrate and 10 parts per million of sodium hydrate. There is no difference between the two crops, and the aluminum hydrate failed to produce any effect upon either. This indicates that, at this stage of growth at least, the reaction of the solution is of more importance than the added plant foods.

Plants were withdrawn from these pans and arranged as in figure *b* to show the effect of the potassium nitrate. This salt gave an alkaline reaction to the solution and produced excellent plants. The "fishhooks" on the roots of the potassium sulphate plants are seen in this plate also (No. 4).

The experiment with aluminum hydrate was repeated with ferric hydrate. This was prepared from ferric chlorid by precipitation with ammonium hydrate and was also washed free from all impurities in order to insure freedom from chlorin and ammonium. A blank culture solution prepared with the ferric hydrate contained less than 0.5 part per million of ammonium. The 5-day-old plants are shown in Plate VII, and when compared with the preceding plates show that the same general characteristics prevail whether calcium carbonate, aluminum hydrate, or ferric hydrate is used to keep down the acidity.

EXPERIMENTS USING CLOVER AND TIMOTHY WITH WHEAT.

Another experiment was made with clover and timothy, using calcium carbonate to neutralize the acidity. The small seeds were sprinkled upon pieces of bolting cloth of a large mesh, placed on the aluminum disks, and grown with some wheat seedlings. It was found impossible to obtain satisfactory photographs of the timothy plants, but both the timothy and the clover behaved in the same way as did the wheat plants, and were even more sensitive to the acids. A few of the clover plants are shown in Plate VIII, figure *b*.

Both with the wheat and the clover cultures the effect of potassium chlorid was not so harmful as that of potassium sulphate. In nearly every case a much better root development was obtained in the potassium chlorid solutions.

While it is a well-known fact that the presence of certain solids by their absorptive action stimulate the growth of seedlings in water culture, the objection to the presence of a solid (calcium carbonate, ferric hydrate, or aluminum hydrate) in one-half of the cultures is overcome by the fact that another solid, carbon black, existed in all alike, and that this solid has as great an absorptive power as either of the others.

It is also clearly evident that a salt, potassium sulphate for example, is not taken into the plant as such, that is, as the molecule (K_2SO_4), but rather as an ion (K). When the potassium sulphate (K_2SO_4) is applied to the soil as a fertilizer it goes into solution and dissociates—that is, splits apart into two radicals, K and SO_4. The potassium is taken up as the ion, while for the time being the SO_4 radical remains in solution and combines with the water to form

PLATE V.

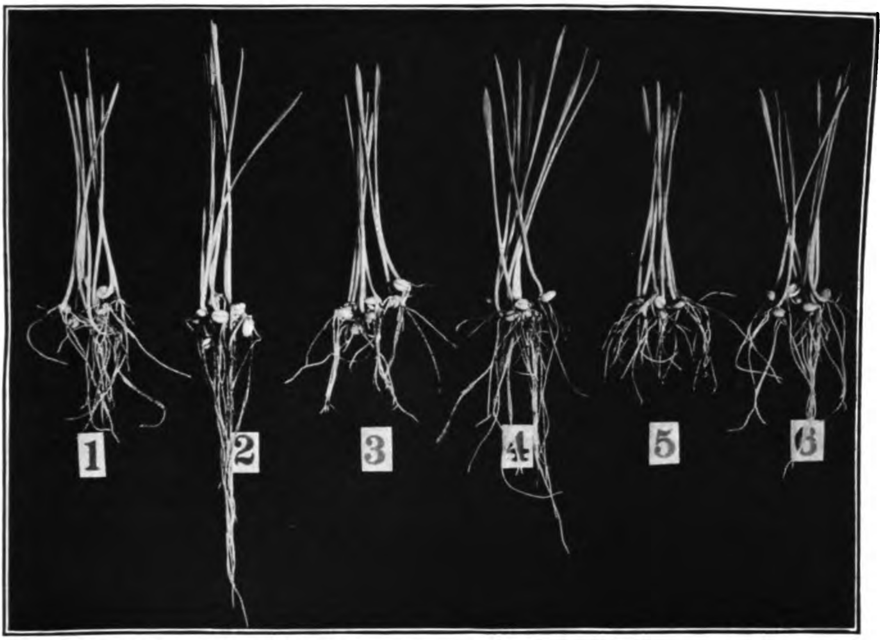

Fig. a.—Crop grown 5 days in (1) distilled water; (2) distilled water + aluminum hydrate; (3) potassium chlorid; (4) potassium chlorid + aluminum hydrate; (5) hydrochloric acid; (6) hydrochloric acid + aluminum hydrate.

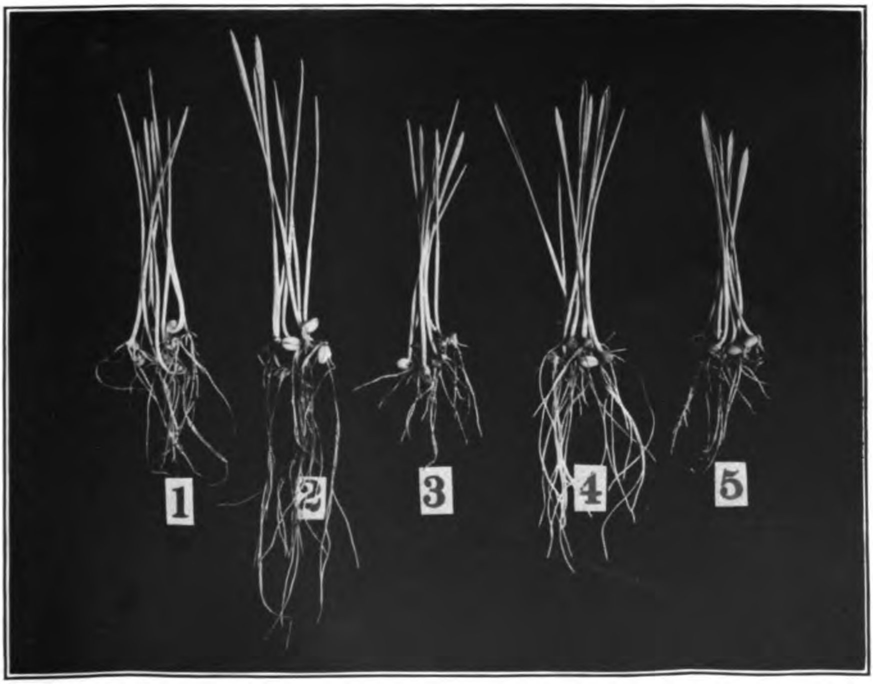

Fig. b.—Crop grown 5 days in (1) distilled water; (2) distilled water + aluminum hydrate; (3) potassium sulphate; (4) potassium sulphate + aluminum hydrate; (5) sulphuric acid.

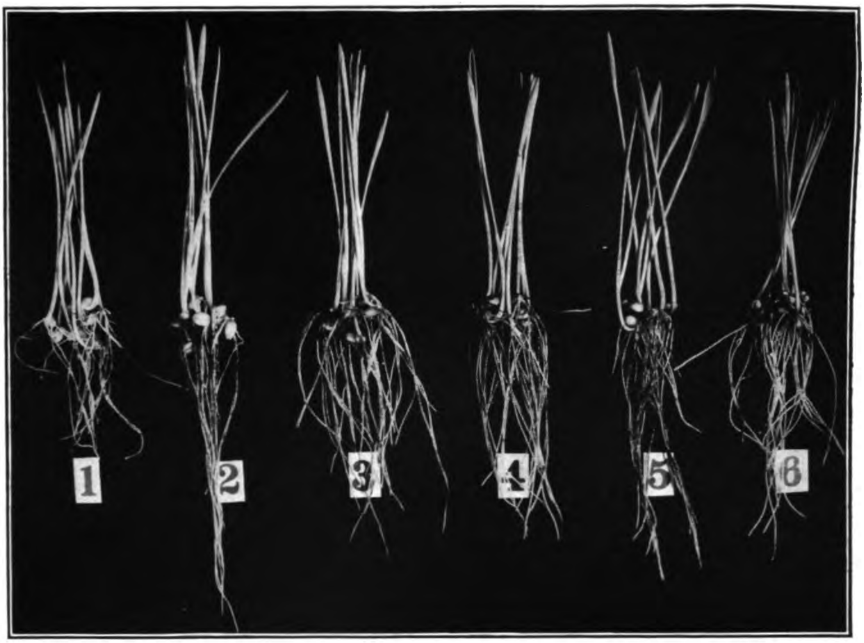

Fig. a.—Crop grown 5 days in (1) distilled water; (2) distilled water + aluminum hydrate; (3) sodium nitrate; (4) sodium nitrate + aluminum hydrate; (5) sodium hydrate; (6) sodium hydrate + aluminum hydrate.

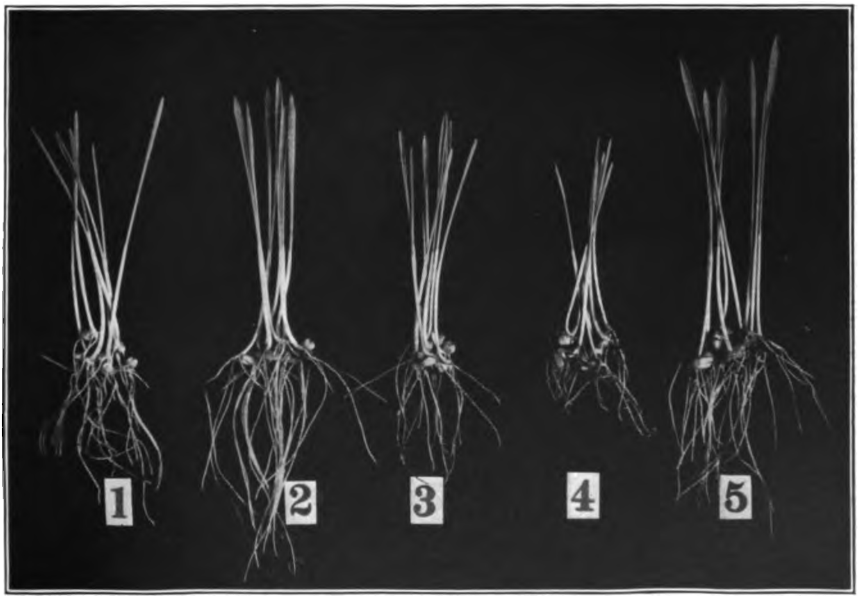

Fig. b.—Crop grown 5 days in (1) distilled water; (2) sodium nitrate; (3) potassium chlorid; (4) potassium sulphate; (5) potassium nitrate.

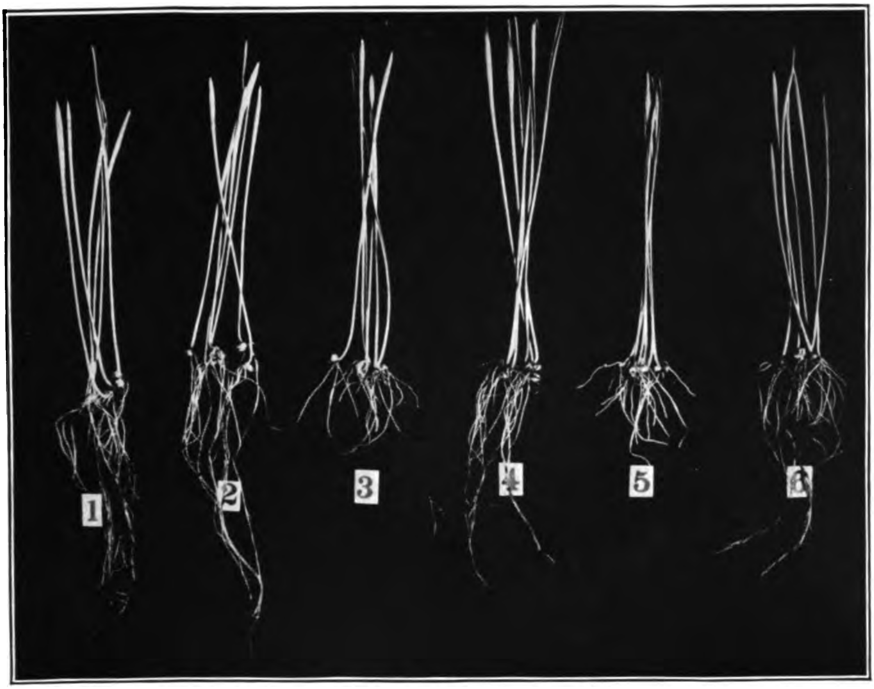

Fig. a.—Crop grown 5 days in (1) distilled water; (2) distilled water + ferric hydrate; (3) potassium chlorid; (4) potassium chlorid + ferric hydrate; (5) hydrochloric acid; (6) hydrochloric acid + ferric hydrate.

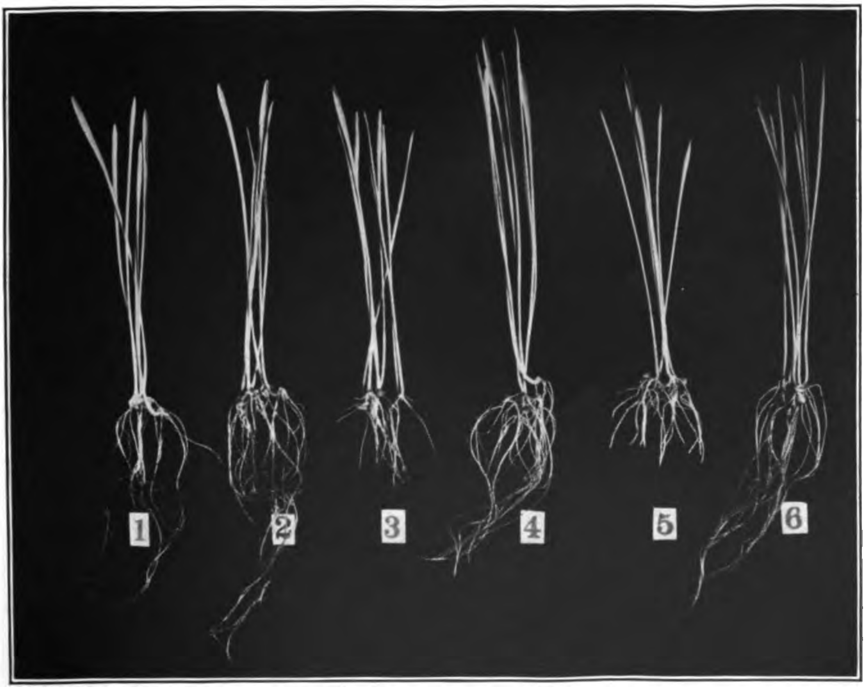

Fig. b.—Crop grown 5 days in (1) distilled water; (2) distilled water + ferric hydrate; (3) potassium sulphate; (4) potassium sulphate + ferric hydrate; (5) sulphuric acid; (6) sulphuric acid + ferric hydrate.

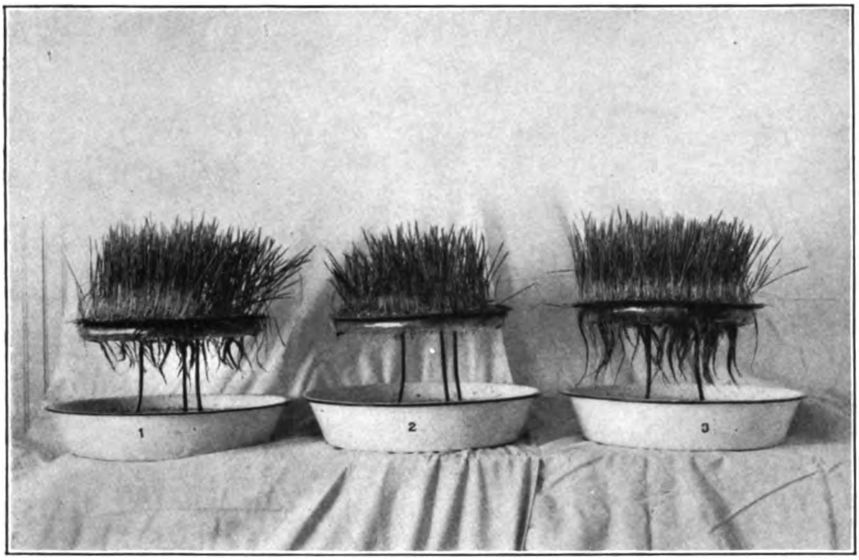

Fig. a.—Comparison of entire pan of wheat plants grown in (1) distilled water; (2) potassium sulphate; and (3) potassium sulphate + calcium carbonate.

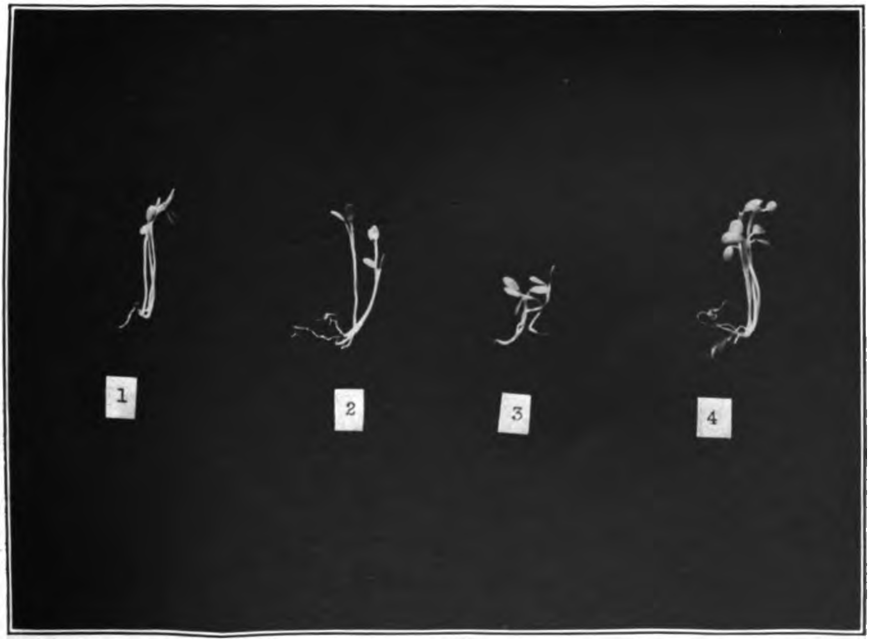

Fig. b.—Clover plants grown with wheat in (1) distilled water; (2) distilled water + calcium carbonate; (3) potassium sulphate; (4) potassium sulphate + calcium carbonate.

sulphuric acid.[1] This is a great secondary source of soil acidity and clearly illustrates the faculty of selective absorption by the plant.

CONCLUSIONS.

The agricultural significance of the physiological study described in this bulletin will be evident from the following discussion. When a soil is in good tilth with an optimum moisture content, each grain is surrounded by a film of water. The plant root comes in contact with this film, and from it draws the water and the mineral salts necessary for growth. Expressed in physical terms, this film is in equilibrium with the soil—that is, if the soil grain contains a silicate of potassium, as feldspar, for example, the water composing the film will bring into the solution about eight parts per million of potash. If some of this potash is removed by the plant more will go into solution, and the concentration of the film will remain fairly constant as long as any feldspar remains in the soil grain.

Although a difference of opinion exists as to how the plant foods are brought into solution, whether by the action of water, by carbon dioxid, or by acids which are exuded by the root tips, all agree that it is absolutely necessary for them to be in solution before they can be taken up by the plant. The soil may, therefore, be considered as a storehouse of reserve material, while the film of soil solution may be considered as a plain nutrient solution in which the plant grows. Whether this soil solution is acid or alkaline is one of the most important factors in plant growth, as has been noted in the foregoing physiological study.

The acidity or alkalinity determines to a great extent not only the particular kind of crop to be grown upon the soil, but also the yield of the crop. No one would think of trying to grow alfalfa in a peat bog or cranberries in a limestone soil. On the other hand, a soil which is naturally alkaline will, under continuous cropping with a rotation containing clover, for example, give smaller and smaller yields as the alkalinity is diminished, until it will be found impossible to grow this legume on account of the acidity of the soil. This phenomenon has been often noted in the limestone regions, particularly in the Shenandoah Valley of Virginia, where soils derived from limestone rocks, and even now having the undecomposed limestone within a few feet of the surface, have become so acid that clover can no longer be advantageously grown. Ground limestone applied to the surface soil will often restore its original fertility.

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[1] In this connection mention should be made of the experiments of Micheels (Action des liquides anodiques et cathodiques sur la germination. Bull. Acad. Roy. Belg., Classe des Sci., 1910, *5:* 391), who studied the effect on wheat seedlings of an electric current produced by a pile of 24 Daniell cells, when passing through various nutrient solutions; and who found that the plants grown near the anode developed roots whose length varied from 10 to 25 mm, while those plants in the cathode solution possessed roots from 140 to 150 mm in length. Similar results were obtained in nutrient solutions containing sodium chlorid, potassium chlorid, potassium nitrate, sodium nitrate, and mixtures of these. A second crop being grown in each of these solutions, even after the electric current was discontinued, similar results were obtained as with the first crop, due to modifications brought about in the solution. From our investigations, the injurious effects noted at the anode, as seen from the length of the roots, could well be attributed to the formation of acid, while near the cathode, the solution being alkaline, the conditions were reversed and therefore favorable for root development.

The heavy clay soils from which bricks are made are always acid, have a high lime requirement, and are usually very unproductive. The oxids of iron exist in such soils in a finely divided colloidal form brought about by an acid condition, and they are usually remedied by an application of lime. The poisonous properties of subsoils also may be due in a large measure to their acid character.

Investigators working with solution cultures and using single salts might do well to take into consideration the reaction of the solutions after the plants have been placed in them. The moment seedlings are placed in a solution of potassium sulphate a rapid absorption of potash begins with a consequent formation of sulphuric acid. This sulphuric acid is a disturbing factor and other substances placed in the solution and kept under observation might owe their apparent beneficial effect to the fact that they simply act as bases, but in themselves have no direct influence upon the plant. In field culture the residual injurious effect of an application of sulphate and chlorid of potash can be overcome by mixing the fertilizer with about twice its weight of lime.

In the experiments here recorded it is shown that the seedlings grown in culture solutions containing potassium chlorid, potassium sulphate, or hydrochloric or sulphuric acid solutions (10 parts per million), exert a selective action whereby the potash ion is absorbed by the roots, while the chlorid or sulphate ion is for the most part left in solution. This causes the solution to become acid, which in turn acts injuriously on the root development.

The addition of lime or iron or aluminum hydrate to culture mediums containing potassium chlorid, potassium sulphate, hydrochloric acid, or sulphuric acid, keeps these solutions alkaline so that they then act favorably on the root development. This would tend to explain why field applications of sulphate or muriate of potash in time render the soil acid,[1] and why the continued use of Chile saltpeter produces an alkaline condition of the soil.

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[1] At the Woburn Experiment Station it has been amply shown that a continuous application of ammonium sulphate renders the soil so acid as to cause the crop to be almost an absolute failure. Adjacent plats, likewise treated with ammonium sulphate, but also given an application of lime, have continued to yield crops as large as are produced by still other plats treated with sodium nitrate only.

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Issued April 22, 1912.

U. S. DEPARTMENT OF AGRICULTURE,

BUREAU OF CHEMISTRY—BULLETIN No. 150.

H. W. WILEY, Chief of Bureau.

TECHNICAL DRUG STUDIES

BY THE

DIVISION OF DRUGS.

Examination of Hydrogen Dioxid Solutions.
By L. F. Kebler, L. E. Warren, and E. A. Ruddiman.

The Purity of Glycerin.
By L. F. Kebler and H. C. Fuller.

Notes on Two Important Alkaloidal Reactions.
By H. C. Fuller.

The Separation and Identification of Small Quantities of Cocain.
By H. C. Fuller.

The Determination of Molybdic Trioxid.
By B. Herstein.

A Method for Testing Ammonium Salts.
By B. Herstein.

Character of Samples of Beeswax Submitted with Bids.
By L. F. Kebler and F. M. Boyles.

WASHINGTON:
GOVERNMENT PRINTING OFFICE.
1912.

LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Chemistry,
Washington, D. C., April 4, 1910.

Sir: I have the honor to submit for your approval a series of technical studies made in the Division of Drugs of this Bureau. Three of these, dealing with hydrogen dioxid, glycerin, and beeswax, are investigations of the quality of these commodities as found on the market, the others are studies of methods of analysis presenting special difficulties, or yielding results which require the greatest degree of accuracy obtainable. I recommend that these papers be published as Bulletin No. 150 of the Bureau of Chemistry.

Respectfully,

H. W. Wiley,
Chief of Bureau.

Hon. James Wilson,
Secretary of Agriculture.

CONTENTS.

Page.

EXAMINATION OF HYDROGEN DIOXID SOLUTIONS. By L. F. Kebler, L. E. Warren, and E. A. Ruddiman 5
- Historical note 5
- Plan of the investigation 7
- Investigation made in Washington 8
  - Determination of hydrogen dioxid 8
  - Preservatives 10
  - Analytical results 11
- Investigation made in Nashville 11
- Comments by manufacturers 11
- Discussion of results 21
  - Hydrogen dioxid 21
  - Acetanilid 21
  - Acidity 22
  - Nonvolatile matter 23

THE PURITY OF GLYCERIN. By L. F. Kebler and H. C. Fuller 24
- Scope of investigation 24
- Discussion of methods and results 25
- Analytical data 26
- Comments by manufacturers 29
- Conclusions 32
- References 33

NOTES ON TWO IMPORTANT ALKALOIDAL REACTIONS. By H. C. Fuller 36

THE SEPARATION AND IDENTIFICATION OF SMALL QUANTITIES OF COCAIN. By H. C. Fuller 41

THE DETERMINATION OF MOLYBDIC TRIOXID. By B. Herstein 44

A METHOD FOR TESTING AMMONIUM SALTS. By B. Herstein 47

CHARACTER OF SAMPLES OF BEESWAX SUBMITTED WITH BIDS. By L. F. Kebler and F. M. Boyles 49
- Introduction 49
- Methods of analysis 49
- Tabulated results of examination 51

TECHNICAL DRUG STUDIES.

EXAMINATION OF HYDROGEN DIOXID SOLUTIONS.

By L. F. Kebler, L. E. Warren, and E. A. Ruddiman.

HISTORICAL NOTE.

Hydrogen peroxid was discovered by P. Thenard in 1818 by the action of dilute acids on barium peroxid in the presence of water. It occurs in minute quantities in the atmosphere and is formed by the oxidation of many substances in the presence of water, for example, phosphorus, zinc, cadmium, tin, bismuth, copper, turpentine, and certain essential oils. It is also generated in appreciable quantities in the metabolic processes of chlorophyll-bearing plants. Hydrogen peroxid was considered a chemical curiosity for many years after its discovery, but was finally introduced as a medicinal agent by B. W. Richardson in 1856. It was believed for a long time by some to be a specific in the treatment of diphtheria, but this is now known to be a fallacy. Hydrogen dioxid is, however, known to be a most valuable germicide and antiseptic and is probably used more generally by the public and the several professions handling medicinal agents than any other single germicide. Numerous investigations have been made to devise methods that would yield a suitable product without the use of preservatives, but the results have not been entirely satisfactory. The experiments undertaken to find a preservative which would minimize the rate of deterioration were apparently more successful.

Numerous reports have been made on the quality and stability of the various brands on the market. The results obtained often were not very favorable, to the regret not only of the manufacturers but the members of the medical profession as well. Some of the early unfavorable results may, however, have been due to the use of new and untried analytical methods. In 1884 Carpenter and Nicholson[a] carefully studied the action occurring between hydrogen peroxid and potassium permanganate in the presence of sulphuric acid. The samples examined by these two workers were represented as containing from 10 to 20 volumes of hydrogen peroxid. The exact amounts of hydrogen peroxid present in the various samples examined were not calculated, but it is plainly evident from the data obtained that there was a great difference between the amount of hydrogen peroxid claimed and the amount actually present.

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[a] Analyst, 1884, 9: 36.

Leffmann and Beam [a] in 1892 published the results of an examination of original packages of a number of brands and their report reflected credit on the material then on the market. The acidity of 50 per cent of the samples, however, was excessive. The number of volumes varied from 9.02 to 15.6. It appears that the potassium permanganate method at the time this investigation was made was fairly well established, as is indicated by the following statement made by Leffmann and Beam: "The proportion of hydrogen dioxid is expressed, as is customary, in volumes of oxygen determined by the standard method with potassium permanganate." Two years later Leffmann [b] examined 21 original packages, in which the amount of available oxygen varied from 1.1 to 11.1 volumes. After making the first determination of available oxygen, the packages were set aside for several weeks at a temperature of 20° C., and then reexamined, the results showing that but little change had taken place. In a number of cases the amount of available oxygen had increased, which is not an uncommon observation, and is due to the fact that the water is more volatile than the hydrogen peroxid. In 1895 [c] Leffmann reported on the quality of hydrogen peroxid used in filling prescriptions and as supplied to the dental profession. Prescriptions calling for 1 ounce were filled by druggists in various sections of the city and samples were also obtained from several dental-supply offices. Leffmann notes particularly the inferior character of the goods supplied to the dental profession.

The most comprehensive examination of hydrogen peroxid made hitherto was reported by Smith and Oerte [d] in 1892, including 50 samples, representing all of the available brands at that time on the market. One-ounce samples in glass-stoppered bottles were purchased in New York City, and in New Haven, Hartford, and Bridgeport, Conn. The first examination was made within 24 hours after the sample was purchased, and the amount of available oxygen found varied from none to 8.86 volumes. These investigators made the following observations on their findings:

> Reviewing the results, it is seen that 56 per cent of the samples contained from 2 to 2.5 grams of the dioxid in 100 cc, or this percentage yielded from 7 to 9 volumes of oxygen. Solutions of this strength, therefore, may be taken as representing the better class of preparations with which we have gained our therapeutic knowledge. That 8 per cent of the samples contained no hydrogen dioxid, and that 36 per cent more must be regarded as deficient, inasmuch as they contained less than 2 per cent of hydrogen dioxid, shows very clearly that more care is needed in dispensing this agent.

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[a] Medical News, 1892, 60: 404.

[b] Medical News, 1894, 64: 288.

[c] Intern. Dental J., 1895, 16: 78.

[d] New York Med. J., 1892, 56: 147.

Tests were also made for boric acid, an agent which at one time was largely exploited as an ideal preservative for hydrogen peroxid.

Dr. E. R. Squibb,[a] in 1892, made an examination of nine samples of prominent brands then on the market. All bore labels claiming 15 volumes strength. The findings, however, show that the actual amount of hydrogen peroxid varied from one-half to two-thirds of that claimed.

The same year Dr. S. S. Wallian [b] published observations on nine brands, determining the amount of available oxygen at intervals. For example, the samples were examined when first received, then reexamined three weeks later and again at the end of six months. The amount of deterioration was marked in several cases, although in most instances the loss in six months was comparatively small. For example, one sample when originally tested showed an oxygen volume of 15, while at the end of six months the amount present was 11.5 volumes. On the contrary, another sample contained when first analyzed 8.25 volumes and at the end of six months only 0.5 volume. The number of volumes stated to be present in the several packages varied from 10 to 15.

R. L. Lloyd [c] reported on five samples of medicinal hydrogen peroxid, which were all represented to be chemically pure and of 15 volumes strength. The examination showed that the amount of available oxygen varied from 10.5 to 13.5, and all of them contained the usual impurities.

H. Endemann [d] in 1894 examined 14 different brands and found the amount of available oxygen to vary from 0.5 to 16.55 volumes. One sample was found to contain 27.35 volumes, but this brand is represented and sold as containing a much higher volume of available oxygen, and for this reason can not properly be classed with the usual hydrogen peroxid sold to the public.

PLAN OF THE INVESTIGATION.

The results obtained by the investigators quoted clearly show that the quality and character of the various brands of hydrogen peroxid were not satisfactory, but during the past 10 years marked improvements have been made in the manufacture of this commodity. It is, however, a common experience that hydrogen peroxid is prone to decompose, and for this reason it was deemed desirable to make a full and exhaustive investigation, with a view, first, to determine the keeping qualities of the various brands of hydrogen peroxid available on the market; second, to ascertain the nature of the preservatives used, if any; third, to establish an age or time limit; and fourth, if possible, to approximate the maximum per cent of variation below the standard prescribed by the Pharmacopœia.

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[a] N. Y. Med. J., 1892, 56: 149.

[b] N. Y. Med. J., 1892, 56: 599.

[c] Amer. J. Pharm., 1893, (4) 65: 276.

[d] Times and Register, 1894, 28: 372.

In order to secure fresh material, the samples were purchased through certain wholesalers, and the results obtained by the first analyses show that they were satisfactory in this respect. In order to eliminate certain personal and climatic factors, it was considered advisable to have examinations made by two observers in separate localities, and Washington, D. C., and Nashville, Tenn., were selected. One hundred and fifteen different samples, representing 36 brands, were examined, the samples including all of the leading makes at present available in the United States. As soon as received they were placed in a cool, partially darkened room, simulating as nearly as possible the conditions usually found in drug stores and other storage places. The temperature in the Washington storeroom varied from 30° C. in the summer to 0° C. in the winter. The samples in the Nashville laboratory were kept under similar conditions, except that the variation in temperature was less marked, ranging from 15° C. in winter to 27° C. in summer.

A list of the brands examined will be found in the table embodying the analytical results (p. 12). Some of the packages bore a guaranty serial number in accordance with section 9 of the food and drugs act and regulation 9 for its enforcement.

As before stated, hydrogen peroxid was once considered a remedy for diphtheria, but it is now known to be virtually worthless for this purpose, yet direct and indirect statements are made to the effect that hydrogen peroxid is a preventative and cure for this disease. It is also represented that the acetanilid is added because of its antiseptic properties. This statement certainly needs confirmation.

INVESTIGATION MADE IN WASHINGTON.

DETERMINATION OF HYDROGEN DIOXID.

The work was begun in December, 1907, some of the initial examinations having been made early in January of 1908. Unless otherwise stated, the methods prescribed by the United States Pharmacopœia, eighth revision, for testing the quality and purity of hydrogen dioxid solutions were employed. In view of the fact that the value of a hydrogen dioxid solution is largely judged by the actual amount of hydrogen dioxid present, the various products were analyzed with particular reference to this point as rapidly as possible after receiving same. The pharmacopœial method was used, excepting that the measured samples were weighed and the percentage computed on this basis. The results obtained will be found in column headed "U. S. P. Method." The results clearly show that a large percentage of the samples examined by this method contain about 3 per cent of hydrogen dioxid. In order to check the permanganate-sulphuric acid method for determining hydrogen peroxid and compare it with other standard methods, it was decided to analyze the samples by three additional methods, namely, the iodin method and the gasometric methods, using sulphuric acid and potassium permanganate in one case and manganese dioxid and sulphuric acid in the other. In each case the hydrogen dioxid solution was measured out and weighed as before stated and aliquots used for the determinations. The iodin method proposed by Rupp[a] was carried out as follows:

Iodin method.—Place 1 gram of potassium iodid in a 250 cc Erlenmeyer flask, add 10 cc of 10 per cent sulphuric acid, and as soon as the salt is dissolved run in 10 cc of the diluted peroxid solution, 1:10, by means of a burette. Cover the flask with a watch glass and allow it to stand for 30 minutes. Then add about 200 cc of distilled water and titrate the liberated iodin with tenth-normal sodium thiosulphate solution, using starch solution as indicator.

This procedure proved somewhat less satisfactory than the pharmacopœial method, being less rapid and giving less concordant results. Each assay was made in duplicate and the mean result is given in the accompanying table in the column headed "Iodin method."

This determination was next made by measuring the volumes of oxygen liberated by means of manganese dioxid and potassium permanganate, respectively, in the presence of sulphuric acid. It was found that Lunge's nitrometer, with a two-way stopcock did not give satisfactory results when the reaction took place inside of the instrument. After making a number of experiments, the following apparatus and procedure were adopted:

Manganese dioxid method.—The apparatus consists of a 50 cc side-arm distilling flask closed with a perforated rubber stopper, through which the delivery stem of a glass-stoppered burette has previously been passed. The side arm of the flask is connected to the nitrometer by means of tightly fitting rubber tubing of any convenient length and securely wired at each end to prevent leakage. The burette is fastened loosely in a Bunsen clamp in such a manner that the burette and flask can be shaken freely in one plane. The nitrometer is filled with water, the level adjusted, and the determination made as follows: Place about 1 gram of finely powdered manganese dioxid in the side-arm flask and add 10 cc of 10 per cent sulphuric acid. Dilute 10 cc of the hydrogen peroxid solution to be tested to 100 cc and place a portion in the burette. After sufficient of the solution is run out to fill the delivery stem of the burette fit the stopper (with attached burette) snugly into the neck of the generating flask, and adjust the level of the water in the nitrometer so as to equalize the pressure within the system. Allow 10 cc of the diluted dioxid solution to run slowly into the generator through the burette. After shaking the flask to liberate the dissolved oxygen as completely as possible again adjust the level and note the temperature and pressure. A correction of 10 cc (the volume of solution run into the generator) is subtracted from the reading, and the remainder reduced to 760 mm pressure and 20° C. temperature.

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[a] Archiv. Pharm., 1900, 238: 156.

The results obtained by this procedure will be found in the column headed "Gasometric analysis, volume of oxygen evolved by MnO_2." The potassium permanganate method was carried out in the manner just described, except that the dilute sulphuric acid and dilute dioxid solutions were placed in a generating flask and sufficient half-normal potassium permanganate solution was added from a burette to give a pink color to the solution. The figures obtained by this procedure will be found in column marked "Gasometric analysis, volume of oxygen evolved by K_2MnO_4," and were made between January 14 and February 12, 1908. The results obtained by the two gasometric methods are fairly concordant, but in general are less reliable than those given by the iodin and official methods, being too dependent on slight variations in temperature, pressure, leakage, and other factors.

In order to determine the rate of decomposition, the samples were reexamined for percentage of hydrogen dioxid by the pharmacopœial method at the expiration of 3, 6, 10, and 12 months after the initial analysis. These results are also recorded in the accompanying table. After determining the percentage of hydrogen dioxid in the various brands by the methods outlined, they were examined for preservatives, acidity, nonvolatile matter, fluorids, arsenic, barium, etc.

PRESERVATIVES.

The principal labels on the packages, as well as the analytical results obtained, show that a large proportion of the hydrogen dioxid solutions contained small quantities of acetanilid. The acetanilid was determined by shaking a 50 cc sample of hydrogen peroxid twice with ether and once with chloroform, combining the several extracts in a tared beaker and drying at 75° C.

Experiments made with samples of hydrogen peroxid containing known quantities of acetanilid showed that this method removed about 95 per cent of the amount present. The acetanilid was identified by its physical and chemical properties, the iodin-phenol reaction proving to be the most satisfactory. Tests were also made for boric acid and borax with negative results.

A number of samples found to be free from acetanilid possessed a noticeable odor of aldehyde after being kept for some time. It was suspected that alcohol had been used as a preservative, but distillation from both acid and alkaline media failed to give a distillate in which alcohol could be detected by the iodoform test. By applying the method directly to the sample, however, good results were obtained. The distillate from these gave the general reactions for aldehyde with fuchsin and sulphurous acid and with Tollen's reagent. The presence of fluorids was indicated in several products, but the results were not conclusive. Caffein was found in several packages of one brand.

ANALYTICAL RESULTS.

The analytical results obtained in this investigation are given in the table on pages 12 and 13, together with a statement of the loss in hydrogen dioxid in six months and in one year on each sample.

INVESTIGATION MADE IN NASHVILLE.

All of the samples were examined as to purity, quality, and strength, by the pharmacopœial methods. The investigation covered two series, one begun in October, 1907, and the other in December of the same year. The acetanilid was extracted by means of chloroform, it having been shown that less than one-fiftieth of a grain could be detected by the phenol-isocyanid reaction. The several samples were reexamined at the intervals of time indicated in the table on page 14.

COMMENTS BY MANUFACTURERS.

The results of the analyses of the several brands were referred to the respective manufacturers, with the following results: No replies were received from the Clarion Chemical Co., Eimer & Amend, Larkin & Scheffer, Lehn & Fink, Matz & Cohen, and Squibb & Sons. The following firms acknowledged receipt of results but offered no criticisms, and their replies are therefore not further considered: American Druggists' Syndicate, Otis Clapp & Son, Harvey Co., Mulford & Co., Norwich Pharmacal Co., H. K. Wampole, William R. Warner, Tilden Co., William S. Merrell, Meyer Bros., Towns & James, and Powers, Weightman, Rosengarten Co.

The comments of the manufacturers who responded are as follows:

ALBANY CHEMICAL CO.

We venture to say that our samples make a very good showing, in view of the fact that hydrogen peroxid loses strength rapidly during the first few months after it has been prepared. What is your experience in this direction?

Acidity . . . The quantity present, found by you, apparently coincides with the findings of our laboratory for several years back, that is to say, our H_2O_2 shows about 50 per cent better than the U. S. P. allows. The same is the case with the solids.

Comment by authors.—It is to be noted that the results show that the acidity is excessive.

BRUEN, RITCHEY & CO.

Your letter of the 16th to hand and in reply would say the hydrogen peroxid we supply conforms to the U. S. P., and the test as outlined by you bears this out with the exception of the test for solids, as the U. S. P. allows only 0.030, whereas you make it much over.

Analytical results obtained in the examination of commercial brands of hydrogen peroxid, Washington, D. C. (Warren).[1]

Name of manufacturer.	Acetanilid.		Acidity (calculated as N/10 H_2SO_4 for 25 cc).	Solids in 20 cc.	Gasometric analysis (volume of oxygen evolved at 20° C., 760 mm) by—		Hydrogen dioxid.						Loss in 6 months.	Loss in 1 year.
	Amount declared (grains per ounce).	Amount found (grains per ounce).			MnO_2.	K_2MnO_4.	Iodin method.	By United States Pharmacopœial method.						
								Initial examination.	After 3 months.	After 6 months.	After 10 months.	After 12 months.		
			cc.	*Gram.*	*cc.*	*cc.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Albany Chemical Co.	$\frac{1}{8}$	0.07	3.50	0.0162	9.85	9.80	2.78	2.85	2.83	2.88	2.70	2.69	+1.05	5.65
	$\frac{1}{8}$	.09	3.60	.0245	9.50	10.00	2.78	2.84	2.78	2.75	2.58	2.53	3.16	10.99
American Druggists' Syndicate.	$\frac{1}{8}$	.14	2.60	.0183	11.17	11.08	3.18	3.28	3.25	3.24	3.14	3.12	1.21	3.25
	$\frac{1}{8}$	.10	2.50	.0180	11.15	11.39	3.20	3.21	3.23	3.23	3.18	3.14	+ .62	2.18
American Peroxide & Chemical Co.	$\frac{1}{16}$	.11	2.10	.0134	10.59	10.67	3.06	3.08	3.08	3.09	3.03	2.99	+ .32	3.05
	$\frac{1}{16}$	.11	2.00	.0158	10.79	10.81	3.05	3.07	3.09	3.08	3.02	2.97	+ .65	3.25
Bene, John.	$\frac{1}{8}$	.14	2.30	.0458	10.70	10.65	3.03	3.07	3.07	3.05	2.98	2.92	.65	4.82
	$\frac{1}{8}$	.13	2.40	.0413	10.69	10.45	3.01	3.07	3.02	3.06	2.96	2.92	.32	4.92
Bruen, Ritchy & Co.	$\frac{1}{8}$	.07	2.70	.0373	10.40	10.40	2.94	2.98	2.98	2.92	2.76	2.68	2.01	10.05
	$\frac{1}{8}$	.08	2.80	.0404	10.45	10.45	2.98	2.98	2.98	2.96	2.70	2.64	.67	11.37
Clarion Chemical Co.	$\frac{1}{16}$	.09	1.50	.0095	11.05	10.80	3.07	3.10	3.09	3.09	2.86	2.81	.64	9.50
	$\frac{1}{16}$	.10	1.45	.0085	10.75	10.51	3.11	3.11	3.12	3.13	3.09	3.04	.64	2.19
Drevet Manufacturing Co.	None.	None.	5.15	.0091	8.15	8.23	2.31	2.31	2.08	1.46	.93	.68	36.79	70.65
	None.	None.	4.95	.0133	8.17	8.07	2.37	2.38	2.11	1.53	.99	.94	35.75	60.44
Eimer & Amend.	None.	.13	2.80	.0324	10.70	10.82	3.03	3.03	3.00	2.99	2.89	2.83	1.96	7.21
	None.	.14	2.60	.0378	11.04	10.56	3.08	3.06	2.99	(2)				2.44
Hance Bros. & White.	$\frac{1}{8}$	.11	2.40	.0392	10.67	10.58	3.02	3.03	2.99	2.97	2.80	2.73	1.98	10.03
	$\frac{1}{8}$	.08	2.90	.0374	10.67	10.67	3.01	3.02	3.02	2.85	2.43	2.38	2.31	21.40
Harvey Co., The G. F.	[3] .0004	.15	2.40	.0142	10.65	10.73	3.05	3.08	3.07	3.08	3.09	2.97		3.41
	[3] .0004	.13	2.40	.0153	10.87	10.38	3.05	3.08	3.09	3.08	3.02	2.98		3.08
Hydrox Chemical Co.	$\frac{1}{16}$	.08	3.60	.0169	10.22	10.57	2.99	3.03	3.02	3.02	2.96	2.84	.33	6.19
	$\frac{1}{16}$	.09	3.15	.0171	10.67	10.40			3.02	3.02	2.92	2.88		5.17
	$\frac{1}{16}$	.10	2.80	.0178	10.36	10.25	3.02	3.04	3.03	3.03	2.90	2.90	.32	4.67
	$\frac{1}{16}$	.08	3.30	.0191	10.30	10.34	3.00	3.01	3.04	2.99	2.91	2.86	.66	5.00
	$\frac{1}{16}$	.15	2.60	.0126	10.33	10.37	3.05	3.08	3.09	3.09	3.02	2.99	+ .32	3.15
	$\frac{1}{16}$	.10	2.70	.0119	10.37	10.47	3.05	3.08	3.08	3.09	3.00	2.97	+ .32	3.41
Lehn & Fink.	$\frac{12}{100}$	.08	3.00	.0404	10.19	10.21	3.01	3.17	3.03	3.03			4.41	
	$\frac{12}{100}$	.06	3.05	.0454	10.58	10.58	3.05	3.09	3.07	3.05	2.96	2.90	.32	6.15
McKesson and Robbins.	$\frac{1}{10}$	.13	3.35	.0182	10.37	10.33	3.04	3.07	3.07	3.00	2.92	2.82	2.28	8.26
	$\frac{1}{10}$	.11	3.00	.0165	10.36	10.38	3.08	3.04	3.02	3.05	2.96	2.91		9.54
Mallinckrodt Chemical Works.	None.	None.	2.15	.2120	10.43	10.10	2.98	3.18	2.96	2.24	.76	.55	29.56	82.62
	None.	None.	2.50	.0234	10.34	10.45	3.01	3.03	3.02	2.97	1.52	.93	1.98	69.26
	None.	None.	2.80	.0253	10.72	10.66	3.00	3.04	3.03	2.99	.43	.29	1.64	90.57
	None.	None.	2.45	.0238	10.22	10.68	3.00	3.00	3.01	2.19	.74	.43	27.00	84.45
Marchand, Charles.	None.	None.	5.50	.0181	13.45	13.45	3.90	3.90	3.78	3.16	2.57	2.40	18.97	38.70
	None.	None.	6.40	.0169	13.67	13.67	3.89	3.93	3.99	3.27	2.67	2.61	16.79	33.51
	None.	None.	6.20	.0164	13.92	13.83	4.02	4.05	4.05	3.21	2.56	2.51	20.74	38.01
	None.	None.	6.90	.0165	13.80	13.77	3.99	4.01	3.81	3.27	2.65	2.50	18.45	37.60

Marchand, Charles (Hydrozone)	None.	None.	10.70	.0165	24.60	24.21	7.04	7.05	6.55	5.78	4.82	4.78	18.01	32.78
	None.	None.	9.30	.0200	24.45	24.74	7.06	7.12	6.73	5.91	4.96	4.61	16.99	35.26
Matz & Cohen	None.	.05	3.85	.0173	9.93	9.91	2.89	2.89	2.89	2.87	2.88	2.69	.69	6.99
	None.	.06	3.00	.0229	9.80	9.97	2.79	2.85	2.89	2.84	2.76	2.73	.70	4.48
Merck & Co	None.	.07	2.20	.0195	10.70	10.74	3.08	3.07	3.10	3.09	2.97	2.91	+ .65	3.75
	None.	.10	2.45	.0114	10.70	10.74	3.05	3.10	3.08	3.09	3.02	3.01	.32	3.00
	None.	.06	2.80	.0124	10.78	10.74	3.05	3.08	3.10	3.09	3.04	3.01	+ .32	2.40
	None.	.13	2.40	.0119	10.60	10.67	3.03	3.09	3.09	3.10	3.02	3.00	+ .32	2.97
Mulford Co., H. K	[4] $\frac{1}{10}$	Trace.	2.20	.0277	10.47	10.55	2.95	3.03	2.97	2.85	2.41	2.20	2.64	27.28
	$\frac{1}{10}$	Trace.	2.75	.0257	10.17	9.65	2.85	2.89	2.84	2.83	2.19	2.04	2.07	29.48
Norwich Pharmacal Co	$\frac{1}{10}$	.06	2.30	.0156	10.40	10.68	2.99	3.02	3.03	3.03	2.93	2.86	.33	5.39
	$\frac{1}{10}$	.08	2.60	.0166	10.16	10.13	3.02	3.04	3.05	3.04	2.97	2.91	.00	4.40
Oakland Chemical Co. (Dioxygen)	None.	None.	1.85	.0094	11.85	11.88	3.56	3.59	3.60	3.58	3.43	3.40	.25	5.37
	None.	None.	2.75	.0086	12.61	12.22	3.57	3.58	3.59	3.54	3.36	3.32	.83	7.10
	None.	None.	2.10	.0091	12.35	12.35	3.49	3.58	3.58	3.52	3.49	3.42	1.67	4.46
	None.	None.	1.70	.0084	12.61	12.34	3.56	3.59	3.64	3.59	3.48	3.41	.00	4.93
Otis, Clapp & Son	$\frac{1}{10}$	.11	2.35	.0147	10.34	10.20	2.94	2.96	2.97	2.98	2.89	2.83	+ .67	4.25
	$\frac{1}{10}$	.11	2.40	.0149	10.33	10.14	2.99	2.97	2.96	2.95	2.88	2.82	.67	4.98
Parke, Davis & Co	$\frac{1}{10}$	.14	3.25	.0109	10.70	10.11	3.01	3.01	3.00	3.00	2.93	2.90	.33	3.55
	$\frac{1}{10}$	.18	4.05	.0111	10.28	10.38	2.98	3.01	2.99	3.01	2.94	2.91	.00	3.35
	$\frac{1}{10}$	.13	3.20	.0114	9.84	9.86	3.02	3.04	3.01	3.01	2.94	2.89	.98	4.66
	$\frac{1}{10}$	.11	3.00	.0129	10.11	10.16	2.93	2.99	2.98	2.99	2.95	2.89	.00	3.76
Powers, Weightman, Rosengarten Co	[5] 0.018	.02	3.20	.0208	10.57	10.40	3.15	3.19	3.12	2.91	2.00	1.72	8.77	46.50
	.018	.02	3.20	.0196	10.68	11.15	3.21	3.20	3.21	3.12	2.68	2.42	2.50	24.52
	.018	.03	4.40	.0186	10.91	10.51	3.15	3.21	3.18	3.12	2.82	2.78	2.80	13.31
	.018	.03	3.20	.0181	11.03	10.71	3.15	3.22	3.16	3.12	2.32	2.11	3.10	34.52
Smith, Kline & French	$\frac{1}{10}$	None.	4.00	.1562	14.68	14.53	4.24	4.27	3.78	2.70	1.72	1.37	36.76	67.86
	$\frac{1}{10}$	None.	3.85	.0929	14.91	14.91	4.28	4.30	4.04	3.38	2.81	2.50	21.39	41.95
Squibb & Sons, E. R	None.	None.	3.80	.0182	10.91	10.71	3.34	3.45	3.37	3.20	2.78	2.74	7.24	20.29
	None.	None.	4.20	.0166	10.91	11.08	3.35	3.41	3.39	3.34	2.41	2.18	2.05	36.24
	None.	None.	3.80	.0175	11.84	11.74	3.30	3.43	3.33	3.11	2.60	2.42	9.32	29.49
	None.	None.	3.90	.0164	11.77	11.19	3.31	3.43	3.31	3.08	2.50	2.29	10.20	33.30
Stearns & Co., Frederick	$\frac{1}{10}$	.13	6.50	.1251	11.08	11.23	3.16	3.17	3.16	3.14	2.99	2.69	.94	15.34
	$\frac{1}{10}$	.12	7.40	.1231	11.00	10.59	3.17	3.18	3.15	3.15	2.98	2.91	.94	8.03
Towns & James	None.	.11	2.70	.0404	10.64	10.64	3.05	3.07	3.08	3.03	2.92	2.88	1.30	6.22
	None.	.10	2.00	.0443	10.86	10.70	3.06	3.06	3.04	3.04	2.95	2.92	.65	4.76
Wampole & Co., H. K	$\frac{1}{10}$	.09	4.50	.0212	11.25	11.27	3.27	3.28	3.30	3.31	3.21	3.18	+ .91	4.39
	$\frac{1}{10}$	.09	4.50	.0195	11.25	11.27	3.27	3.29	3.30	3.30	3.20	2.75	+ .30	16.36
	$\frac{1}{10}$	.09	2.00	.0115	10.00	10.17	2.95	3.01	3.04	3.02	2.95	2.89	+ .33	2.13
Warner & Co., Wm. R	$\frac{1}{10}$	.05	3.05	.0135	10.11	10.18	2.91	2.95	2.94	2.89	2.78	2.69	2.03	9.04
	$\frac{1}{10}$	.05	3.20	.0102	10.78	10.59	2.95	3.01	2.99	2.95	2.80	2.74	1.99	8.92
	$\frac{1}{10}$	.10	2.70	.0126	10.57	10.70	3.09	3.02	3.05	3.04	2.98	2.91	+ .66	3.54

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[1] The pharmacopœial standard is as follows:
"* * * Should contain, when freshly prepared, about 3 per cent, by weight, of absolute hydrogen dioxid, corresponding to about 10 volumes of available oxygen." Twenty-five cubic centimeters should not contain more free acid than that equivalent to 2.5 cc of tenth-normal sulphuric acid; 20 cc should contain not more than 0.03 gram of solids. For other data see United States Pharmacopœia, 8th revision, p. 55.

[2] Bottle exploded between March 31 and June 30.

[3] No denomination given on label.

[4] Maximum.

[5] Per cent.

Analytical results obtained in the examination of commercial brands of hydrogen peroxid, Nashville, Tenn. (Ruddiman).

Name of manufacturer.	Acetanilid.		Acidity (calculated as N/10 H_2SO_4 per 25 cc).	Total solids in 20 cc.	Per cent of hydrogen dioxid present at the end of—									Per cent of loss in 5 months.	Per cent of loss in 9 months and in 1 year.
	Declared.	Found.			October.	November.	December.	January.	March.	May.	July.	September.	October.		
Investigation begun in December, 1907:	*Grains per ounce*	*Grains per ounce.*	*cc.*	*Grams.*											*In 9 mos.*
Albany Chemical Co.	⅛	Present	3.9	0.0211	......	......	2.84	2.87	2.79	2.75	2.68	2.60	......	3.13	8.3
American Drug Syndicate	⅛	do	2.7	.0161	......	......	3.32	3.31	3.28	3.26	3.24	3.23	......	1.80	2.6
American Peroxide & Chemical Co.	1/16	do	2.9	.0116	......	......	3.11	3.11	3.08	3.07	3.05	3.03	......	1.28	2.5
John Bene	⅛	do	3.0	.0411	......	......	3.15	3.12	3.08	3.05	3.00	2.99	......	3.17	5.0
Bruen Ritchy & Co.	⅛	do	3.2	.0330	......	......	3.04	3.02	2.98	2.91	2.86	2.74	......	4.27	9.7
Clarion Chemical Co.	1/16	do	1.5	.0079	......	......	3.16	3.15	3.13	3.11	3.09	3.08	......	1.58	2.4
Drevet Manufacturing Co.	None.	None	4.9	.0112	......	......	2.42	2.29	2.07	1.79	1.42	1.16	......	26.03	51.8
Eimer & Amend	None.	Present	3.1	.0394	......	......	3.11	3.08	3.05	3.01	2.97	2.95	......	3.21	5.4
Hance Brothers & White	⅛	do	3.3	.0365	......	......	3.10	3.08	3.01	2.94	2.83	2.72	......	10.90	12.2
Harvey Co., The G. F.	[1] 0.0004	do	2.9	.0092	......	......	3.18	3.15	3.13	3.11	3.09	3.08	......	2.20	3.3
Hydrox Chemical Co. (Hamlin's Peroxide of Hydrogen)	1/16	do	2.9	.0109	......	......	3.14	3.11	3.10	3.07	3.05	3.04	......	2.23	3.2
Lehn & Fink	0.12	do	3.4	.0423	......	......	3.11	3.11	3.06	3.04	2.99	2.96	......	2.25	4.9
McKeeson & Robbins	1/16	do	3.3	.0142	......	......	3.13	3.11	3.06	3.04	2.98	2.90	......	2.87	7.3
Marchand, Charles (hydrozone)	None.	None	9.5	.0173	......	......	7.27	6.98	6.35	5.99	5.21	4.59	......	17.60	36.8
Marchand, Charles (glycozone) [2]	None.	do	......	......	......	......	0.00	......	......	......	......	......	......	......	......
Matz & Cohen	None.	Present	5.1	.0157	......	......	2.89	2.88	2.87	2.85	2.81	2.79	......	1.38	3.6
Mulford Co., The H. K.	1/10	Trace	2.0	.0260	......	......	2.88	2.85	2.76	2.66	2.43	2.21	......	8.68	23.1
Norwich Pharmacal Co.	1/16	Present	2.1	.0140	......	......	3.08	3.06	3.03	3.02	2.99	2.96	......	1.94	3.8
Otis, Clapp & Son	1/16	do	2.6	.0129	......	......	3.02	3.00	2.98	2.95	2.93	2.92	......	2.31	3.4
Smith, Kline & French	1/16	do	5.6	.3036	......	......	4.68	4.50	3.93	3.25	2.49	1.88	......	30.55	62.0
Frederick Stearns & Co.	1/32	do	7.6	.1188	......	......	3.23	3.22	3.19	3.15	3.06	2.98	......	2.47	7.8
Towns & James	None.	do	3.3	.0380	......	......	3.11	3.11	3.09	3.02	2.98	2.92	......	2.90	6.2
H. K. Wampole Co.	1/16	do	4.0	.0164	......	......	3.45	3.43	3.42	3.38	3.35	3.32	......	2.03	[3] 3.7
Investigations begun in October, 1907:															*In 1 year.*
Hydrox Chemical Co.	1/16	do	2.5	.0101	3.09	3.06	3.06	3.05	3.01	2.99	2.95	2.87	2.86	2.86	7.2
Larkin & Scheffer	None.	None	2.8	.0930	1.93	1.91	1.88	1.84	1.74	1.60	1.42	1.27	1.21	9.84	37.4
Mallinckrodt Chemical Works	None.	do	2.6	.0160	2.94	2.94	2.89	2.80	2.43	2.02	1.63	1.37	1.27	17.34	56.9
Marchand, Charles	None.	do	6.6	.0242	.08	.06	.05	.04	.03	.02	.01	.01	0.00	50.00	98.6
Merck & Co.	None.	Present	2.7	.0133	3.09	3.09	3.09	3.09	3.06	3.03	2.99	2.97	2.95	.97	1.2
The Wm. S. Merrill Co.	⅛	Small amount	4.0	.0320	2.92	2.89	2.79	2.69	2.32	1.85	1.33	.98	.79	20.54	72.7
Meyer Brothers	0.142	Present	3.0	.0308	2.98	2.97	2.95	2.93	2.89	2.84	2.78	2.72	2.68	3.02	9.8
Oakland Chemical Co.	None.	None	2.4	.0105	2.36	2.30	2.20	2.14	1.92	1.60	1.20	.87	.74	14.40	68.9
Parke Davis Co.	1/16	Present	2.8	.0162	2.75	2.75	2.74	2.73	2.69	2.68	2.62	2.55	2.47	3.27	10.1
Powers, Weightman & Rosengarten Co.	0.018	None	1.3	.0268	2.55	2.45	2.35	2.27	2.05	1.75	1.21	.76	.62	19.60	75.7
Squibb & Sons	None.	do	7.8	.0136	2.56	2.51	2.47	2.43	2.28	2.10	1.86	1.65	1.65	10.93	30.5
The Tilden Co.	1/16	Trace	2.5	.0203	2.97	2.97	2.91	2.82	2.34	1.90	1.52	1.22	1.13	21.21	62.0
Warner & Co., Wm. R.	None.	None	2.9	.0193	.32	.30	.28	.26	.23	.20	.16	.14	.12	28.12	63.4

[1] No denomination given.

[2] Represented and believed to contain hydrogen dioxid, but in fact does not.

[3] Loss.

We can account for this by your not using a desiccator, for if it is dried any other way it will take moisture from the air, thereby greatly increasing the residue.

We have never put out any peroxid which runs higher in residue than the U. S. P. allows.

Comment by authors.—All moisture determinations were made by cooling the dried material in a desiccator.

THE DREVET MANUFACTURING CO.

* * * We note the analyses of our various preparations made by your department, and are absolutely at a loss to understand the results obtained, unless perhaps the samples used were very old.

As you probably know, we were about the first to manufacture the medicinal peroxid of hydrogen, and we have jealously guarded the high standard of our preparations for more than 30 years and beg of you the privilege of submitting for your careful examination samples of our preparations taken from our present stock on hand, and at the same time we would kindly ask you to allow us to offer certificates of analyses made by well-known and prominent chemists of this city for your consideration.

We would be pleased to have your representative call and collect personally these samples from our stock if you wish, or we would be glad to forward such samples to you.

Trusting you will grant our request, and assuring you of our earnest desire and efforts to place our goods on the market in full conformity with or even superior to the U. S. P. standard, we beg to remain.

HANCE BROTHERS & WHITE.

We received yours of the 16th ultimo on the 28th. At the same date we inclosed it to Mr. John Bene, Brooklyn, N. Y., from whom we procure our supply of peroxid hydrogen. We have examined the articles several times and found it to comply so closely with the U. S. P. requirements, and in view of its perishable nature and its use almost entirely for external purposes, have issued it as being equal to other makes.

We are herewith inclosing Mr. Bene's reply, which rather confirms our estimate of his article and shows his faith in the declaration on his invoice of its conforming to the requirements of the Federal food and drugs act.

The letter of Mr. John Bene referred to reads as follows:

Your letter of the 28th instant to hand with inclosures, and in reply the hydrogen peroxid I supply you conforms strictly to the U. S. P., and the test as outlined by Dr. Wiley bears this out with the exception of the test for solids, as the U. S. P. allows only 0.030, whereas he makes it much over. I can account for this by his not having used a desiccator, for if it is dried any other way it will take moisture from the air, thereby greatly increasing the residue. I have never put out any peroxid which runs higher in residue than the U. S. P. allows.

HYDROX CHEMICAL CO.

We hasten to acknowledge receipt of your analyses of Hydrox peroxid of hydrogen, Hamlin's peroxid of hydrogen, and American peroxid of hydrogen, all of which products are manufactured by the chemists of the Hydrox Chemical Co.

We believe it is pretty well demonstrated that acetanilid, $\frac{3}{16}$ grain to the fluid ounce, acts as a preservative to this preparation (as is demonstrated by your analyses) so that the last drop in any container is full U. S. P. strength, making the preparation thoroughly dependable in the hands of the practitioner at all times.

We beg to state that in our investigations we have found that the breaking up or decomposition of acetanilid causes a slight increase in acidity of the product, this change taking place probably three to six months after its manufacture, but not a sufficient increase to render the product harmful.

We have also observed that peroxid of hydrogen when not properly stored in an upright position decomposes, either with or without acetanilid, by contact with corks; foreign matter being taken up by the preparation.

We are pioneers in the manufacture of permanent peroxid and wish to make our product conform as closely as possible to governmental requirements; at the same time believe our present standard is so high as to permit of little change in formula.

MALLINCKRODT CHEMICAL WORKS.

Replying to yours of the 16th instant, I assume that the copy of analyses of our hydrogen peroxid refers to samples taken from a lot supplied by us in accordance with the requirements of your department—that it contain no acetanilid or other preservative. It is well known that hydrogen peroxid solution will not keep without the addition of some preservative, and we are not surprised that the samples should have lost so largely in the percentage of strength after being kept for a period of one year.

You are doubtless also aware that the acidity test of the United States Pharmacopœia is unreliable and shows larger amounts of acid when none is present, the amount being dependent on the manner of manipulation.

If you will examine our hydrogen peroxid as we supply it to the trade, with acetanilid as a preservative, I think you will find that if kept with ordinary precaution it will lose very little, if any, strength during the period of a year.

Comment by authors.—The samples examined in Washington did not contain any acetanilid, but the presence of caffein was established. The samples, furthermore, were not taken from the lot supplied, at the request of the department, free from acetanilid. The sample examined in Nashville was purchased through a local wholesaler. The point, therefore, raised relative to the absence of acetanilid as a preserving agent is not substantiated.

CHARLES MARCHAND.

* * * I have sold my interests in the Drevet Manufacturing Co. the beginning of January, 1909, and I have not manufactured "Marchand's Peroxide of Hydrogen," "U. S. P. Peroxide of Hydrogen," "Hydrozone," or "Glycozone" since that time.

Under these circumstances, I have nothing whatever to do with the goods manufactured and sold by the Drevet Manufacturing Co., although they are still labeled "Prepared only by Charles Marchand."

McKESSON & ROBBINS.

* * * We do not see that there is any comment for us to make upon your results, as the results speak for themselves. We might, however, add that we have recently adopted a time-limit label similar to the inclosed sample, which appears on all packages of peroxid sent out by us. While your results show that our peroxid will keep almost unchanged for a year, we place the time limit at six months, owing to the uncertainty surrounding the keeping properties of this preparation under the varying conditions met with in different parts of the country.

In our opinion six months would be a reasonable time limit for all manufacturers to adopt both for the protection of the public as well as for the reputation of the manufacturer and retailer. * * *

Comment by authors.—The time limit adopted by this firm is in accordance with the findings of this investigation.

MERCK & CO.

* * * We have looked over your tabulated results with interest. From the tabulation which you offered it appears that in the acidity test the method of the United States Pharmacopœia has been employed. But do you not think this method rather unreliable? This question was raised about three years ago by numerous investigators.

Replying to your favor of the 13th, asking us to advise you as to what we consider a reliable test for determining the amount of free acid in pharmacopœial hydrogen peroxid, we would say that we obtain the most accurate results by the use of methyl orange indicator instead of the phenolphthalein indicator prescribed by the United States Pharmacopœia.

Uniform and accurate results may also be obtained by simply titrating the original solution of dioxid with decinormal potassium hydroxid, using methyl orange and omitting all evaporation and heat.

Comment by authors.—The observations noted are in accordance with our findings.

OAKLAND CHEMICAL CO.

Replying to your letter of December 16, received yesterday, we beg to thank you for it. The plan that you are using of notifying manufacturers of the results of your investigations and the opportunity you are offering for comment on the report is very fair and commendable.

May we ask you to kindly give us the numbers which are stamped with a rubber stamp on the labels of the bottles which you have been using as samples? We ask this information so that we can check up your results with our records. We take it that this will not be unsatisfactory to you, and it will certainly be a matter of satisfaction to us to make the comparison. * * *

We beg to acknowledge receipt of your favor of the 5th instant. None of our records show anything that would compare with your report on this sample. The uniformity of the other four and the relative closeness of your statement with our results justify the assumption that the report on sample No. 5 can not be accepted as indicating either an exceptional case or a fair average of quality of our product; the not unreasonable possibility of something unusual having happened to this sample either before or after it reached your hands, the further possibility that it may not actually be our product, though apparently an original bottle bearing our label, are sufficiently important to warrant our request that this sample be either eliminated or a full opportunity be given us to make a rigid investigation that would allow us to trace its history completely. * * *

Your favor of March 1 was duly received. The statement that the label on the package of dioxogen, on which there is some question, contains the number 91, and which statement had previously been made in a department letter of January 13, has been carefully reviewed, and we beg to make you the following report.

The number referred to, which is placed on these bottles, is the key by which we can identify each lot of our product, its date of manufacture, and all the records which refer to it. These numbers run in series. The present series of numbers began in March, 1907, and No. 91 of this series was reached December 15, 1908. If the bottle bearing the mark received by your department from ——— Co. was obtained in October, 1907, it could not have belonged to this series started in March, 1907, and must have belonged to a previous series.

The previous series began in November, 1904, and No. 91 of this series was reached August 13, 1906. In August, 1906, ——— Co. received a shipment of goods bearing this number. Consequently this bottle, which your department has reported on, had been in ——— Co.'s possession one year and two months before you received it During the fall of 1906 and spring of 1907 we removed our manufacturing plant from Brooklyn, N. Y., to Richmond County, N. Y., and when our manufacturing operations began at the new plant we started a new series. It was during the confusion of this period that this lot, from which the bottle your department has examined came, was made.

Under these circumstances, we respectfully request you to eliminate this test from your published report. If it is the purpose of this report to supply information as to the quality and character of goods of the different manufacturers, then we respectfully point out that a bottle of a product of the character of peroxid of hydrogen, which is over a year old before the test is made, is not fairly illustrative of average conditions or quality. If other tests which you have made from time to time indicate that such a variation as this particular sample shows might be reasonably expected, we withdraw the request.

If in your judgment it is reasonable to expect that goods would be more than one year old before they reach the consumer's hands, as was this sample, then we withdraw the request.

If you in your experience with our product have found anything that would indicate that the result of this sample conveys a true estimate of the actual quality of dioxogen, we withdraw the request.

Comment by authors.—Considerable care was exercised to secure fresh samples. There was nothing on the package in this case to indicate possible deterioration from age. With justice to all, no one sample could be eliminated, but the explanation offered by the manufacturer is given in full.

PARKE DAVIS & CO.

* * * On the whole we feel very much pleased to note a statement from the Bureau of Chemistry which so nearly confirms our claim that we are marketing a high-grade hydrogen peroxid and one which is remarkably stable, considering the nature of the substance.

The amount of acetanilid reported is in each instance somewhat less than the quantity originally added to the solution of peroxid at the time of manufacture. Our formula calls for the addition of three-sixteenths grain of acetanilid per liquid ounce of the peroxid solution, this being equivalent to 0.41 gram per 100 cc. In each case the amount reported by you is less than this. It is entirely possible that in some cases a portion of the acetanilid has been destroyed by the action of the hydrogen peroxid, particularly in the old samples.

In examining the proportion of acid reported for the five different samples we note that this is in every instance high. We presume that the figures given are the direct statement of the acid in terms of cubic centimeters tenth-normal acid in 25 cc of the hydrogen peroxid, and are consequently not an indirect statement of the number of cubic centimeters of tenth-normal acid required to titrate back, as directed in the Pharmacopœia. If our first supposition is correct, we beg to say that the amount of acid reported by you is in every instance undoubtedly excessive in that you have estimated not only the amount of free acid present, but have also estimated as well the acid produced from the acetanilid which has decomposed by heating with alkali in carrying out the test as stipulated in the Pharmacopœia.

We have had occasion to refer in one or two previous instances to this error in estimating acid by the official method in hydrogen peroxid preserved by the use of acetanilid. Theoretically the acid in three-sixteenths grain of acetanilid will neutralize or is equivalent to 0.75 cc of tenth-normal potassium hydroxid. In actual practice, however, we find by making numerous tests upon a given specimen of hydrogen peroxid, to a portion of which we add three-sixteenths grain acetanilid per ounce and to the second portion of which no acetanilid is added, that this three-sixteenths grain of preservative will increase the seeming content by about 1.3 cc of tenth-normal acid. We can not altogether account for this increase above theory, but presume that it is due to some acid body produced by the decomposition of the acetanilid, produced by the action of the hydrogen peroxid on the acetanilid in the presence of the alkali in carrying out the evaporation.

Turning now to the five samples reported, if we make a correction in each instance for the amount of acetanilid reported by you, the equivalents would be as follows:

	cc N/10 KOH.
Sample No. 1	0.6
No. 2	0.7
No. 3	0.5
No. 4	0.45
No. 5	....

Subtracting the above quantities of tenth-normal acid from the quantities you report, the free acid would appear as follows:

	cc.
Sample No. 1	2.65
No. 2	3.35
No. 3	2.7
No. 4	2.55
No. 5	....

Making the theoretical correction, each of these samples would fall below the requirements of the U. S. P. for the content of free acid with the exception of No. 3.

We do not regard this matter of slight excess of free acid as being of very great consequence, but think it best to call your attention to this error, inasmuch as we presume you will go into this matter of hydrogen peroxid very thoroughly, and the evidence which we offer is the result of a very great deal of experience. There is really no reason for resorting to the indirect method as outlined in the Pharmacopœia. Equally good results and (in the case of hydrogen peroxid containing acetanilid) more correct results are obtained by direct titration. It is only necessary to add to 25 cc of the hydrogen peroxid about 5 or 6 minims of phenolphthalein test solution (U. S. P.) and titrate directly with tenth-normal alkali to the development of a light-pink color. Under the conditions this method is accurate to 0.1 to 0.2 cc. For practical purposes the test in the Pharmacopœia should be stated as follows:

If to 25 cc of the solution of hydrogen peroxid 5 or 6 minims of phenolphthalein test solution are added, not more than 2.5 cc tenth-normal KOH should be required to produce a pink color (limit of free acid).

Comment by authors.—It is probably true that the presence of acetanilid will increase the acidity of hydrogen peroxid, particularly when determined according to the prescribed method, but the manufacturer is aware of the facts and should, therefore, make due provisions. If he desires to add acetanilid, and it is known to him that the addition of this chemical will increase the acidity of the product, he should make ample allowance to come within the maximum limit of the prescribed acidity. The subject of direct titration, using phenolphthalein as indicator, is discussed on page 22.

SMITH, KLINE & FRENCH CO.

We beg to acknowledge receipt of your letter of the 16th with analysis of our hydrogen peroxid.

First of all we note that samples 1 and 3, judging from their strength, must be our 15-volume peroxid. Therefore, so far as acidity and solids are concerned, they would have to be judged from a different standpoint than the 10 volume, for it must be remembered that 15-volume peroxid, being one-half stronger, the acidity and the solids would necessarily increase in the same proportion.

This peroxid is plainly branded 15 volumes, and is therefore distinguished from the 10-volume product.

We are surprised to note that only sample No. 3 contains acetanilid, because we have been putting acetanilid in our peroxid for almost two years, and this would indicate that samples 1 and 2 are very old. If you could let us know the numbers on the packages we could in turn let you know the age of the peroxid, which might be of interest. * * *

We beg to acknowledge receipt of your letter of January 7 giving us the serial numbers of packages of peroxid, the test of which you have reported under date of December 16.

We find that the No. 4926 was put up on October 31, 1907, and No. 4935 on November 11, 1907. The packages, therefore, were two years old last October and November. It is therefore fair to presume that they were a year old before the tests were begun.

Since that time peroxid in general, and our peroxid in particular, have been much improved. We think it would be only fair, when the tests are published, to give the date of manufacture and the date of the beginning of the test, because otherwise it would be rather unfair to, for instance, say 15-volume peroxid, and then give the strength found, unless the interval between the manufacture of the goods and the date of the first test was given. We leave this, of course, entirely to your own good judgment.

Comment by authors.—It will be observed that the investigation was begun in October, 1907, and the samples, examined were, therefore, comparatively fresh.

FREDERICK STEARNS & CO.

We are very much surprised to learn that you have found any of our product to run high in solids and so very high in acidity. We test very carefully every batch of this preparation and aim to have it comply with pharmacopœial requirements, except in so far as we, like many other manufacturers of this article, add acetanilid as a preservative, as is stated on every label. This would increase the amount of total solids by the amount of acetanilid so added. In addition to this, the acetanilid also has the effect of increasing the apparent percentage of acid because in the U. S. P. method of assay, the peroxid solution is boiled with the tenth-normal KOH which saponifies the acetanilid, some of the alkali combining with the acetic acid. On a 25 cc sample, basing calculation upon the amount of acetanilid you found (26.5 mg=average), we ought to deduct 0.5 cc tenth-normal KOH from the total number of cubic centimeters required to neutralize the acid, because this amount (0.5 cc) is equivalent to the 26.5 mg acetanilid present in a 25 cc sample of peroxid.

We understand, of course, that this does not explain the high results you obtained, but it occurred to us that this matter may not have been taken into consideration.

We have gone over our record of tests for the entire year of 1909 and find that we had but four batches that yielded as much as 30 mg of residue, the next highest was 26 mg the average for the year being 20.73 mg. Likewise the average acidity for all of the hydrogen peroxid manufactured during the entire year is 2.31 cc tenth-normal KOH, tested according to the U. S. P.

We always make our determinations before the addition of the acetanilid.

Comment by authors.—Manufacturers are aware that the Pharmacopœia prescribes a maximum for solids, and therefore if a preservative is added it should be only in such amounts as to keep well within the limits.

DISCUSSION OF RESULTS.

HYDROGEN DIOXID.

As before stated, the value of a solution of hydrogen peroxid is largely judged from the percentage of hydrogen dioxid present. The investigations were begun in October, 1907, and continued over approximately one year. The results obtained on hydrogen dioxid by the gasometric methods and the iodin method do not materially vary from those by the pharmacopœial method, and for that reason the latter will be made the basis of comment. The Pharmacopœia states that hydrogen peroxid solution should contain, when fresh, about 3 per cent by weight of absolute hydrogen dioxid, corresponding to about 10 volumes of available oxygen.

A review of the results obtained by the first examination shows that 21 of the samples fall below the 3 per cent basis and 8 fall below a 2.75 per cent basis. The results obtained five and six months after the first examination show that 24 of the samples examined had lost 10 per cent and over of the original content of hydrogen dioxid, while on a 15 per cent basis, 21 were found defective, which is only 3 less than on the 10 per cent basis. The point may be raised that this commodity is less stable in warm than in cold weather, but this generally accepted idea is not supported by the data. The degree of deterioration taking place between 5 and 6 months and 9 and 10 months, respectively, which covers the summer period, does not differ materially from that noted over similar periods of time in cooler weather. It should, moreover, be noted that these observations were made after the samples were at least five or six months old and deterioration had set in, a condition favorable for decomposition.

Knowledge of trade conditions leads to the belief that it is not necessary to keep hydrogen peroxid solutions on hand longer than five or six months, the latter being the maximum limit.

ACETANILID.

The results of this work undoubtedly show that acetanilid retards the deterioration of hydrogen peroxid solutions. The inhibition, however, is not uniform. For example, of two samples of hydrogen peroxid, made by the same firm and both containing acetanilid, one will deteriorate rapidly, while the other is fairly stable. The cause of such apparent abnormalities has not yet been determined. There are probably numerous contributing factors, but one undoubtedly is the difference in the methods of manufacture. Furthermore, the absence of acetanilid from a number of brands and their slow rate of deterioration shows that acetanilid is not necessary to prevent or retard decomposition. Hydrogen peroxid solutions containing acetanilid sooner or later develop a yellowish or brownish tint and an odor resembling nitrobenzol. To what extent the presence of nitrobenzol and similar agents are objectionable has not been established.

The Pharmacopœia neither recognizes nor prohibits the use of acetanilid as a preservative or antiseptic or otherwise, yet many packages bear labels declaring the presence of acetanilid and at the same time designate the article to be the pharmacopœial product, an obvious inconsistency. The acetanilid is not added because of its antiseptic property, as is indicated on some labels, but because of its apparent inhibiting influence on deterioration. The presence of acetanilid was declared in 72 samples, and found in 70.

ACIDITY.

The results on acidity show that a large majority of the products are excessively acid. This may be interpreted that the standard equivalent of 2.5 cc of tenth-normal sulphuric acid for 25 cc of the solution is too exacting. By placing the upper limit at 3 cc of tenth-normal sulphuric acid for 25 cc, 51 samples would still be excessively acid, and by placing the standard at 4 cc, 22 would remain above the limit.

A certain amount of the acidity may be due to the oxidation of the acetanilid present. This is a point, however, which should be provided for by the manufacturer who uses the chemical. The maximum acidity prescribed by the United States Pharmacopœia does not appear to be too exacting, particularly when it is remembered that hydrogen peroxid solutions are largely used as mouth washes. If this product should contain an excessive amount of free acid, it would of necessity exert a deleterious influence upon the teeth.

Numerous experiments have been made on the determination of the acidity of hydrogen peroxid solutions by the pharmacopœial and other methods, and it has been found that methyl orange could be substituted with advantage for phenolphthalein if the present pharmacopœial method is retained. Experience and experiments furthermore show that the free acidity can readily be determined by direct titration, using phenolphthalein as indicator, and there seems to be no good reason for continuing the use of the present pharmacopœial method.

NONVOLATILE MATTER.

The results show that a comparatively small number of samples contain an excessive amount of solids. There should be little difficulty in properly adjusting this matter, particularly in view of the fact that the majority of those containing excessive amounts of solid material come from the same factory. One manufacturer states definitely that the amount of nonvolatile matter is in excess of that prescribed by the Pharmacopœia. The solid material on examination was found to contain one or more of the following: Chlorids, phosphates, sulphates, and acetanilid. In no case was the presence of fluorids established, but in several instances a trace of metals was noted. Tests made on the solid material show that the acetanilid was neither completely decomposed nor volatilized at 120° C.

Notwithstanding the fact that hydrogen peroxid solutions are known to be more or less unstable, some manufacturers place such statements as the following upon the labels: "Permanent," "Guaranteed unsurpassed in keeping qualities," "A permanent * * * preparation," "Unsurpassed in strength and keeping qualities," "Full 10 volumes strength," etc. The analysis showed that the sample bearing the latter label was less than two-thirds of the standard strength. Why such statements should be made in connection with products of this character is difficult to understand, since it is well known that they are liable to deterioration. It should be noted, however, that this investigation shows the quality of medicinal hydrogen peroxid available on the market to be, on the whole, very satisfactory.

THE PURITY OF GLYCERIN.

By L. F. Kebler and H. C. Fuller.

SCOPE OF INVESTIGATION.

The question of the purity of glycerin has assumed great importance during recent years because of the extensive use of this product in the preparation of medicinal agents. This has been impressed on the manufacturers to such an extent that the quality of glycerin on the market has been greatly improved and the best grades may now be procured without difficulty.

The claim has often been advanced that the present glycerin standard is too rigid and in certain respects can rarely be attained; hence the Pharmacopœia should be so modified that it will be consistent with conditions which actually exist. To confirm or refute this statement it was necessary to investigate the quality of the glycerin on the market in the United States, and to determine exactly to what extent samples from different sources conformed to the tests prescribed in the Pharmacopœia. Another purpose was to learn whether there were any glycerins which would not reduce Fehling's solution, the claim that no such article was obtainable being of importance owing to the use of glycerin in the preparation of Haines's solution, an alkaline copper liquid which has been recommended for detecting sugar in urine and whose value as a testing reagent would naturally be impaired if reduction occurred when no sugar was present.

Letters were sent to manufacturers in different parts of the country requesting samples, the scope of the inquiry being outlined sufficiently to give them every opportunity to submit glycerin of as high quality as possible, together with any comments and suggestions deemed desirable. When the examination had been completed, the several dealers who had responded were sent analyses of their own makes of glycerin, with a request for further criticisms before the publication of the results. Samples were received from the following 11 firms in the United States, two additional samples of foreign manufacture having been purchased on the open market:

Armour Soap Works, Chicago (two samples).
Colgate & Co., New York.
Harshaw, Fuller & Goodwin Co., Cleveland, Ohio.
Wm. A. Jobbins, Aurora, Ill.
Jas. S. Kirk & Co., Chicago.
Larkin Co., Buffalo, N. Y. (two samples).
Marx & Rawolle, New York.
Peet Bros. Manufacturing Co., Kansas City, Kans.

Powers, Weightman, Rosengarten Co., Philadelphia.
Procter & Gamble Co., Cincinnati, Ohio.
M. Werk Co., Cincinnati, Ohio.
E. Schering, Berlin, Germany.
Price's Patent Candle Co., London, England.

DISCUSSION OF METHODS AND RESULTS.

The samples were examined first according to the methods prescribed in the Pharmacopœia. A rigid examination was made for arsenic in order to determine whether even traces of this substance were left in the glycerin; special tests were made to note the action on Fehling's solution, and a sample of Haines's solution was made up with each product. The physical appearance was good in every instance. The samples were all neutral to litmus paper. The specific gravities at 25° C. varied from 1.248 to 1.258, in most cases being above 1.250, with an average of 1.254. In this respect they were all above the standard requirement of 1.246 at 25° C.

The pharmacopœial test for sugar gave negative results, as would be expected. Experiments were then conducted, noting the action on Fehling's solution, using concentrated and diluted glycerin and without previously heating with acid as in performing the sugar test. Two samples which reduced Fehling's solution in the undiluted state gave no reduction on dilution; two reduced the solution on diluting, but did not in the concentrated condition; three showed no reduction either diluted or undiluted; while the remainder reduced Fehling's solution under any condition. Several of the samples which reduced Fehling's solution in the undiluted condition had reduced ammoniacal silver nitrate in the Hager test.[a] Haines's solution, when made according to directions given in works on urinalysis[b] was reduced in every instance, thereby substantiating the claims which had already been advanced in this connection.[c]

Most of the samples were well within the requirements as to the color imparted by concentrated sulphuric acid, various shades of yellow occurring. One sample was dark brown and fluorescent, while another turned brown and gave off a strong fatty odor. In fact, the odor varied more than the color, some samples having the fatty odor just mentioned, while others had an unmistakably pleasant aromatic smell; four had a characteristic hydrocarbon odor, and in one instance the product was practically without color or odor, but evolved a quantity of minute gas bubbles. When the samples were treated with alcohol and sulphuric acid and warmed on the steam bath ethereal odors were noted in all cases, the unpleasant butyric ester predominating, although with two samples an agreeable fatty odor was noticed. In certain instances this test ran parallel with the former one, in that samples which gave scarcely any color or odor with sulphuric acid gave but a slight ethereal odor, but for the most part the tests were entirely at variance, as will be noted on examining the table on page 27. When simply heated on the steam bath marked differences in odor were noted, four samples gave off a slight odor of no particular character, two an aromatic odor, and the remainder a fatty odor of varying intensity.

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[a] Handbuch der Pharm. Praxis, 4th ed., 1905, 1: 1221.

[b] Purdy, R. G., Practical urinalysis and urinary diagnosis. 1895, p. 103.

[c] Mayer, J. L., The reducing action of glycerin, Merck's Report, 1905, 14: 165.

A reaction was obtained showing a slight trace of sulphates in five products, a trace of chlorids in two, and a reduction of silver nitrate in five, this being very marked with one specimen; oxalates were indicated in two samples from the same manufacturer; heavy metals were shown in three cases, while six gave no reactions for metals or acids.

Only one sample showed the presence of objectionable amounts of arsenic, and this was of foreign make and labeled "arsenic free." The Marsh test was rigidly applied to every glycerin in order to determine how completely the arsenic had been eliminated; six specimens gave no mirror, and the others, with the exception of the one above mentioned, contained less than one part in a million. It is of interest to note that of two samples submitted by one manufacturer one contained arsenic and the other did not.

Satisfactory results seemed impossible with the pharmacopœial test using ammoniacal silver nitrate; reduction took place in every instance, and it was apparent that no significant conclusions could be drawn from its use. Accordingly it was abandoned for the test described by Hager,[a] with which better comparative results were obtained. Five cubic centimeters of the sample were mixed with 5 cc of 26 per cent ammonia water and 5 drops of silver nitrate added; the mixture was allowed to stand at the ordinary temperature in the dark for 15 minutes and its appearance noted. There was no reduction of the silver nitrate in six cases, and a marked reduction in two, the other samples being slightly colored.

ANALYTICAL DATA.

The investigation showed that glycerin could be produced which would answer all of the pharmacopœial requirements with the exception of the ammoniacal silver nitrate test. It also showed that although some glycerins gave off a fruity odor when treated with alcohol and sulphuric acid, they were otherwise of good quality; therefore the wording of this test should be modified in order to include the disagreeable butyric odor as it now discriminates only against a "fruity odor."

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[a] Loc. cit.

Glycerin analyses.

Manufacturer and serial number.	Odor on heating.	Odor with alcohol and sulphuric acid.	Color and odor with sulphuric acid.	Action with silver nitrate and ammonium hydroxid (Hager's test).	Metals and acids.	Arsenic (parts per million).	Haines's solution (cuprous oxid formed).	Undiluted and treated with Fehling's solution.	Diluted (1-1) and treated with Fehling's solution.	Diluted (1-3) and treated with Fehling's solution.	Specific gravity at 25° C.
Armour, 2129...	Fatty.....	Unpleasant ethereal.	Slight yellow; fatty.	Colorless......	None..........	None.	In one week.	No reduction hot or cold.	In one hour cold; no reduction hot.	No reduction hot or cold.	....
Armour, 2130...	...do......	.....do.........	Dark yellow; fatty.	.....do.........	.....do.........	None.	In three hours.	No reduction cold; little on heating.	.....do..........	.....do..........	....
Colgate, 2134....	Slightly aromatic.	Ethereal......	Yellow; slight fatty.	Slight coloration.	.....do.........	0.17	A little overnight.	Slight reduction.	In half an hour cold; no reduction hot.	.....do..........	1.258
Harshaw, Fuller, & Goodwin, 2132.	Strong fatty.	Ethereal with butyric compound predominating.	Light yellow; aromatic.	Very slight color.	Trace of sulphate.	0.25	Overnight	No reduction hot or cold.	.....do..........	Slight reduction cold; none hot.	1.253
Jobbins, 2137...	Slight fatty.	Unpleasant ethereal.	Very little......	Colorless......	Slight trace of sulphate.	0.2	A little overnight.	Slight reduction.	Slight reduction. cold; none hot.	No reduction hot or cold	1.255
Kirk, 2138......	Very little.	Fatty ethereal.	Darkens; odor of machine oil.	Reduces considerably.	Slight trace of sulphate and chlorid.	None.	At once...	Reduces at once hot; reduces in one-half hour cold.	Reduction in 15 minutes both hot and cold.	.....do..........	1.256
Larkin, 2139....	Slight; not fatty.	Fatty ethereal (turns dark).	Brown - fluorescence; oily.	Slight color....	Slight oxalate; reduces with silver nitrate.	0.4	...do......	No reduction hot or cold.	No reduction hot or cold.	.....do..........	1.254
Larkin, 2140....	Considerable.	Fatty ethereal (turns dark).	Yellow; oily...	Yellow.........	Slight oxalate; reduces with silver nitrate; slight color with H_2S.	None.	...do......	Turns green cold; no action hot.	.....do..........	.....do..........	1.255
Marx & Rawolle, 2136.	Slightly fatty.	Slightly ethereal.	Little color; air bubbles.	Colorless......	None..........	0.4	Overnight	No reduction hot or cold.	.....do..........	.....do..........	1.248
Powers-Weightman - Rosengarten, 2131.	Strong aromatic.	Unpleasant ethereal.	Dark yellow; fatty.	Very slight color.	.....do.........	0.75	...do......	Little on heating.	Considerable cuprous oxid comes out overnight cold; no reduction hot.	.....do..........	1.255
Procter & Gamble, 2135.	Slightly fatty.	.....do........	Little color; slight fatty odor.	Colorless......	Trace of sulphate and chloride.	None.	...do......	No reduction hot or cold.	No reduction hot or cold.	.....do..........	1.250

Glycerin analyses—Continued.

Manufacturer and serial number.	Odor on heating.	Odor with alcohol and sulphuric acid.	Color and odor with sulphuric acid.	Action with silver nitrate and ammonium hydroxid (Hager's test).	Metals and acids.	Arsenic (parts per million).	Haines's solution (cuprous oxid formed).	Undiluted and treated with Fehling's solution.	Diluted (1-1) and treated with Fehling's solution.	Diluted (1-3) and treated with Fehling's solution.	Specific gravity at 25° C.
Werk, 2133.....	Fatty.....	Ethereal with butyric compound predominating.	Slight yellow; fatty.	Very slight color.	None..........	None.	Little cuprous oxid overnight.	Reduces on heating; none cold.	Cuprous oxid in one-half hour cold; no reduction on heating.	No reduction hot or cold.	1.254
Schering, 2192...	Slight.....	Slight fatty ethereal.	Slight yellow; slight fatty.	Considerable reduction.	Slight reduction with silver nitrate; slight iron.	0.4	Reduction over night.	Reduction hot and cold.	Reduction hot and cold.	Reduction hot and cold.	1.257
Price, 2193......	Strong fatty.	Strong fatty ethereal.	Yellow brown; considerable fatty.	Little color....	Much reduction with silver nitrate; slight iron.	3.75	Considerable reduction overnight.	.....do.........	.....do.........	.....do.........	1.256
Peet Bros., 2818.	Slight.....	Slightly fruity; not disagreeable.	Yellow; slight hydrocarbon, oily.	Colorless......	Trace of sulphate reduces with silver nitrate.	Slight.	....	Slight reduction hot; none cold.	No reduction hot or cold.	No reduction hot or cold.	1.2522

COMMENTS BY MANUFACTURERS.

Replies were received from all of the manufacturers whose goods were examined. The following excerpts from these letters are of interest. This investigation shows that the glycerin available on the American market is of excellent quality, the one sample which was materially defective being of foreign origin.

COLGATE & CO.

We would call attention to the fact that the directions for Fehling's test as given by the U. S. P. are not explicit enough, as it is not stated whether the mixture is to stand for six hours at ordinary temperature or on the water bath. The test is entirely uncalled for, as any C. P. glycerin should leave but a very slight residue seldom over 0.01 per cent on being allowed to burn off spontaneously, and if any sugar were present it, as well as other nonvolatile impurities, would show in the amount of residue. Silver tests, and ammoniacal silver tests, are unreliable, as it is easy to get results ranging from no coloration to a heavy mirror of silver on the same glycerin by slight variations in the method of testing. The test is no criterion for the presence of acrolein, as it is easy to work the test so as to give a heavy silver mirror with any glycerin made, although acrolein may be entirely absent. I will add that the sample of glycerin we sent you gave no appreciable reduction when we tested it with ammoniacal silver nitrate U. S. P.

The mixing of glycerin with an equal volume of concentrated sulphuric acid is another test which is apt to give very erratic results.

In reference to specific gravity it should be mentioned that the specific gravity given in the U. S. P. as corresponding to 95 per cent glycerin is not correct within 1 per cent of glycerin, but was calculated from old, inaccurate tables. Of the published tables, that of Nicol at $\frac{20° \text{C.}}{20° \text{C.}}$ is most accurate, being very nearly correct.

Now that the U. S. P. has become the legal standard, it is desirable that the tests should be rational in every particular, and that they should be so rigid as to exclude inferior goods, and on the other hand should require no unnecessary tests and no tests apt to give erratic results.

Comments by the authors.—The application of the designation "C. P." to the available glycerin misrepresents the facts and should therefore be discontinued.

THE HARSHAW, FULLER & GOODWIN CO.

Under date of May 18, 1907, we forwarded to you a sample of our glycerin, which represented the average run of goods we were at that time turning out. This, however, was nearly two years ago, and since that time we have been constantly endeavoring to improve our product. We have succeeded so far as sulphate is concerned. Your analysis of the sample referred to states that it showed a trace of sulphate. An analysis of our present product would not show this.

With reference to butyric acid, it seems impossible to entirely eliminate this. In operating our glycerin refinery, we must purchase our crude material from a great number of producers located in France, Italy, Spain, Germany, South America, and elsewhere. No two of them make exactly the same product. There are certain producers who make such an excellent quality of crude that it is possible for us to produce from it a refined glycerin without any objectionable features. The quantity of this material, however, is comparatively small, and while we could send you a sample made from it, which would test as above, yet it would not fairly represent an average product.

WILLIAM F. JOBBINS, (INC.).

Definite, fixed limits of impurities should be prescribed and taste and odor tests eliminated, as two different individuals almost invariably have a widely different sense of taste or smell. One chemist will sometimes be of opinion there is a foreign taste or smell, while another can detect neither.

As the glycerin industry throughout the world has been revolutionized by our patented processes and machinery, which have gradually been adopted during the last past 15 years, and are now almost exclusively used by glycerin producers, we submit we speak as recognized authorities on the subject, and beg to suggest that the Pharmacopœia requirements be changed, as follows:

Specific gravity not less than 1.258 at 15° C. (60° F.), the specific gravity to be ascertained by means of a Westphal specific gravity balance, all materials being at the temperature of the room, the value found being reduced to the value at 15° C. by adding a product represented by (temperature − 15) × 0.000581.

Carbonaceous residue, including mineral and readily carbonizable impurities, not to exceed 0.009 per cent. Test: Weigh 50 grams of glycerin in a tared platinum dish, heat cautiously until it inflames, then remove the source of heat (preferably Bunsen burner) and allow the glycerin to burn away in a place free from draft; transfer the dish to a desiccator and when cool weigh.

Ash, including chlorids, not to exceed 0.005 per cent. Test: Ignite the carbonaceous residue at a red heat till it ceases to lose weight; then transfer to a desiccator and when cold weigh.

Chlorids, not to exceed 0.001 per cent, figured as NaCl. Test: Put the ash in 100 cc distilled water, make slightly acid with HNO_3, add two or three drops of a cold, saturated solution of neutral chromate of potassium as indicator, then run in from accurately calibrated burette one-tenth normal silver nitrate volumetric solution till tinge of permanent red color appears. The cubic centimeter of one-tenth normal silver nitrate volumetric solution used multiplied by 0.005806 for each cubic centimeter, and then by 2, giving the percentage of chlorin as sodium chlorids.

Total acid equivalent, in terms of NaOH, not to exceed 0.02 per cent. Test: Weigh 100 grams of the glycerin, add 100 cc of distilled water and a few drops of phenolphthalein solution as indicator. Run in an excess of one-tenth normal NaOH solution (3.976 grams of NaOH to 1 liter) from a burette, boil for three minutes over Bunsen burner, then titrate with tenth normal H_2SO_4 solution (4.8675 grams per liter) run in from a burette, until pink color just disappears. The NaOH solution used, less the H_2SO_4 solution used, must not exceed 5 cc to neutralize.

In addition to the foregoing tests an aqueous solution (1 cc of glycerin to 10 cc of distilled water) with 5 cc of tenth-normal silver nitrate volumetric solution, the mixture shaken and placed in a dark place for 10 minutes, may assume a slight pink or gray tinge, but must not turn red nor black, nor give a precipitate. The glycerin shall then be considered chemically pure and free from chlorids, acids, or other impurities.

These tests cover all possible impurities to be found in C. P. glycerin, and being set forth with definiteness leave no latitude for controversy between chemists, and insure a glycerin of such purity as is applicable in the use of medicines, foods, or drugs, and are requirements which no honest refiner of glycerin can possibly make objection to, and are, in fact, the tests by which the leading glycerin refiners are governed.

American glycerin is equal to any foreign glycerin. Regarding test with Fehling's solution, we have to say that same is no longer employed. If adulterated with sugar it would increase the carbonaceous residue beyond the recognized limit, so sugar adulteration is provided against by the carbonaceous residue test.

J. C. LARKIN CO.

In looking back over our tests we find one made on the batch of glycerin from which the sample forwarded you was taken. We find that this test indicates the glycerin to be very much better quality than your tests show. Of course, the matter of odor, darkness of color when mixed with sulphuric acid, etc., is largely a matter of judgment, and the tests are not specific enough to enable two independent operators to check exactly their results. For instance, what you might consider a strong odor, or a dark color, might not be so considered by our chemist, etc.

We note, however, that you state that this glycerin reduces ammoniacal silver nitrate when tested by the U. S. P. method. We never turned out a glycerin, as far as we are aware, that responded to this test. We are making tests of our glycerin continually as we produce it, and looking back over our files we can not find a single test which shows a quality as poor as your tests would indicate. We would call your attention to the fact that, as far as we can learn, the sample of the glycerin which you have tested is almost two years old. We can not tell what happened to it during the interval after it left our hands and was tested by you.

PEET BROS. MANUFACTURING CO.

It is barely possible that our C. P. glycerin contains a trace of sodium chlorid, but it is almost inconceivable that it should contain a trace of sulphate, as we use muriatic acid exclusively in its preparation, with the resultant conversion of any sulphate which might be present into chlorids.

Also notice you mention a slight trace of arsenic. We would like to ask what test was used in arriving at this conclusion?

POWERS, WEIGHTMAN, ROSENGARTEN CO.

Referring to your favor of the 10th instant, regarding the report of examination by the Department of Agriculture on our sample of glycerin, we would advise that some of our tests agree with those made by the department, but vary in others as follows:

Test 3: D. A.—Strong aromatic odor on heating at temperature of water bath. P. W. R.—Slight odor of volatile fat acids on heating at water bath temperature.

Test 4: D. A.—Unpleasant ethereal odor on warming with alcohol and sulphuric acid. P. W. R.—Pleasant ethereal odor on warming with alcohol and sulphuric acid.

Test 5: D. A.—Dark yellow color with strong odor when mixed with equal volume of sulphuric acid in the cold. P. W. R.—Dark yellow color and mild odor when mixed with equal volume of sulphuric acid without heating (the mixture becomes hot when mixed). Pale yellow and faint odor when mixture is kept cold with water.

Test 6: D. A.—Reduces ammoniacal silver nitrate U. S. P. test. P. W. R.—This test is doubtful. No reaction reported in two tests out of three.

Test 7: D. A.—Slight color with ammoniacal silver nitrate, Hager's directions. P. W. R.—No reaction. Hager's directions rather vague; there may be a reaction under some conditions of applying the test.

Test 10: D. A.—Reduces Fehling's solution in the cold on standing, no reduction on heating. P. W. R.—No reduction, either hot or cold.

Test 11: D. A.—Reduces Fehling's solution made according to Haine; sugar none. P. W. R.—Does not reduce Fehling's solution made according to Haine.

Of course the same sample was not used in both instances, which doubtless accounts for some of the differences shown above. However, both tests seem to agree that the glycerin contains the merest trace of a fat acid, which most likely is butyric.

PRICE'S PATENT CANDLE CO. (LTD.).

We may say at once that the results differ so widely from those obtained from the examination of our current make, all of which is carefully tested before issue, that we are quite at a loss to understand the divergence.

SCHERING & GLATZ.

* * * We had lengthy correspondence with Messrs. Schering in 1905 and 1907, relative to their glycerin, and they reported that their product complies with every test of the U. S. Pharmacopœia, save that for fatty acids. This test they consider extravagant and impossible to comply with, therefore labeling their product, as per specimen inclosed: "Schering's glycerin, thrice distilled, complies with every test of the U. S. Pharmacopœia, excepting the one for volatile fatty acid, which is not made by the pharmacopœia of any other country. While this test may show the presence of minute quantities of fatty acids, these are so infinitesimal that they are wholly without significance. No glycerin, so far as we know, responds to this test."

Reverting to your esteemed communication of the 10th ult., we would say that the Chemische Fabrik auf Action (vorm. E. Schering) in Berlin, beg to comment as follows on your findings relative to their glycerin:

"*Slight odor on heating at temperature of water bath.*—It is not stated that the odor noticed was an unpleasant one. Doubtless most products will show an odor upon warming, no matter how pure and how odorless in the cold state.

"*Slight fatty ethereal odor on warming with alcohol and sulphuric acid.*—Minimal traces of fatty acids are contained in every distilled glycerin. Our repeated request to send us any glycerin which is absolutely free from fatty acid, you have not been able to comply with.

"*Yellow color with slight fatty odor when mixed with an equal volume of sulphuric acid in the cold.*—A yellowish coloration on mixing glycerin with concentrated sulphuric acid is unavoidable and has been declared admissible by the U. S. Pharmacopœia.

"*Traces of heavy metals.*—It may be conceded that our glycerin contains traces of heavy metals. So long as vessels made of heavy metals are used, this will not be preventable. But the method of test of the U. S. Pharmacopœia is of such a nature that discoloration upon addition of hydrogen sulphid may also be due to the presence of organic substance, or the glycerin itself.

"*Arsenic.*—It has not been found possible in our analytical laboratory to demonstrate in unequivocal manner the presence of arsenic in our chemically pure glycerin. But if the finding of '0.4 part per million' is correct, the glycerin abundantly fulfils the rovisions of the U. S. P. on this point.

"*Reduces with ammoniacal silver nitrate, U. S. P. test and Hager's directions.*—Regarding the behavior of ammoniacal silver solution to glycerin a great deal has been published, but the matter is still unclear. Inasmuch as refined glycerins, which are far less pure than distilled glycerins, do not reduce, the test is generally not considered of importance.

"*Reduces with Fehling's solution.*—It would be interesting to learn how this test was made. Inasmuch as Dr. Wiley's analysis showed no sugar and as we ourselves know that none is present, we do not understand the object of the test. That glycerin is a readily oxidizable body and therefore has a reducing action, must, of course, not be overlooked."

CONCLUSIONS.

It appears from this investigation that a high grade glycerin should meet the following requirements:

Its specific gravity should be about 1.250 at 25° C.

It should be neutral to litmus paper.

It should leave no ash on ignition.

It should give off but a slight odor when heated alone at the temperature of the water bath.

It should not give off an unpleasant ethereal odor nor a strong fruity odor when warmed with alcohol and sulphuric acid.

It should not develop more than a yellow color when mixed with an equal volume of sulphuric acid and no disagreeable odor should be evolved.

When treated with silver nitrate and ammonium hydroxid, according to Hager, no color, or at most only a yellow color, should develop.

It should give no reaction for sulphates, chlorids, oxalates, or metals.

It should give no reaction for sugars.

When diluted with an equal volume of water it should give no reduction with Fehling's solution.

It should not contain arsenic in excess of the limit prescribed in the Pharmacopœia.

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NOTES ON TWO IMPORTANT ALKALOIDAL REACTIONS.

By H. C. Fuller.

During the systematic analysis of a large number of pharmaceutical products it has been apparent from time to time that certain of the standard reactions employed as a means of identification of alkaloids can not be relied upon absolutely and in all circumstances. The tests in question are the Vitali color reaction given by atropin and the oxidation reaction of sulphuric acid and potassium bichromate on strychnin. When the ingredients of certain medicines were separated, and tests subsequently applied in order to establish the identity of the unknown substances, residues were found to give a purple color with alcoholic potash very characteristic of atropin, yet neither atropin nor belladonna extract had been added to the preparation, and in other instances on treating a residue with sulphuric acid and potassium bichromate a characteristic purple color was obtained, yet neither strychnin nor nux vomica was found. These facts become especially important in forensic work where, in many instances, the analyst has a comparatively small sample at his disposal, and often the proportion of alkaloidal substances is not large.

Allen[a] says in connection with Vitali's reaction:

> The violet reaction is almost peculiar to atropin and its isomers. Out of some 60 alkaloids examined none others were found to give a violet coloration. Strychnin gives a red, brucin a greenish, and homatropin a yellow color when similarly treated,

while Menegazzi[b] holds that strychnin acts similarly to atropin.

Beckmann[c] states that on evaporating veratrin with nitric acid a yellow residue is left which on the addition of alcoholic potash gives a violet color that quickly changes to a cherry red and gradually becomes brown.

Allen, commenting on the sulphuric-acid bichromate test, states that "colocynth resin gives a very similar reaction to strychnin, but is readily detected by agitating the acidulated solution with benzene or ether."

The similarity of the action of the alkaloids of gelsemium and of berberin has been mentioned in the literature from time to time.

In order to determine which of the drug extracts in common use yield substances responding to the reactions in question, a series of experiments was conducted with some of the ordinary drug products employed in the manufacture of galenical preparations. It was deemed advisable to work with the drug itself and not with the purified alkaloid, as the former is generally used in the class of products under consideration. An alcoholic extract of the drug was evaporated cautiously until the alcohol had been driven off. The residue was then digested with normal sulphuric acid and filtered into a separator. To remove the basic constituents, ammonium hydroxid was added in excess and the solution shaken three times with Prolius mixture. The solvent was filtered and evaporated, and the residue dissolved in normal sulphuric acid. The solution was filtered into a separator and shaken out, first with chloroform and then with petroleum ether. Ammonia was then added in excess and the solution shaken out successively with three portions each of petroleum ether (boiling at 30° to 50° C.) and chloroform. The solvent solutions were washed with water and then filtered and evaporated. The residues thus obtained were practically free from any material other than that of an alkaloidal nature, and were virtually of the same character as those obtained in making a systematic analysis of a medicine. Portions of these residues were treated, according to the customary methods of analysis, with nitric acid and, after evaporation, with alcoholic potash; also with sulphuric acid and potassium bichromate.

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[a] Commerical organic analysis, 1907, vol. 3, pt. 2, p. 258.

[b] Ber. d. chem. Ges., 1894, 27 (4):275.

[c] Archiv. pharm., 1886, 224; :481.

In the case of the Vitali reaction, working with the portion removed by the petroleum ether, the addition of alcoholic potash to the dry residue gave a purple color in the case of extracts of belladonna, coca, nux vomica, and yohimbe. The material obtained from aconite, cinchona, colchicum, colocynth, conium, gelsemium, gentian, hydrastis, ipecac, opium, physostigma, pilocarpus, sanguinaria, and Veratrum viride gave no reaction of this character. The portion removed by the chloroform gave a purple color in the cases of belladonna and yohimbe, but not with nux vomica or coca. The color with nux vomica was very red. Colchicum, though the bulk of the alkaloid had been previously taken out of acid solution by chloroform, gave a residue which showed a faint purple tint, and cinchona a pale purplish pink.

In the case of sulphuric-acid bichromate reaction the petroleum ether residues from nux vomica, gelsemium, and yohimbe gave a purple color. The chloroform residues from the same drugs and from hydrastis acted similarly. Colocynth yielded a substance which developed a pinkish purple on standing, but it did not resemble the usual strychnin test. The reaction in the case of colocynth was obtained with a sample which had previously been shaken three times with chloroform in acid solution. The extract from sanguinaria gave a pale purple color visible in thin layers. The residue obtained from opium gave a slight purple color most prominent in thin layers. No similar reactions were obtained with any of the other drugs mentioned.

These facts are, as previously stated, especially important when only small quantities of material are available, and further complications arise when mixtures are under examination. The similarity of the colors given by the coca and belladonna alkaloids is of special interest inasmuch as they are often found together. However, if cocain is present it will be at once apparent by the characteristic odor of ethyl benzoate, which is given off on the addition of the alcoholic potash, and, furthermore, the physiological effect on the tongue, together with the microscopic examination of its well-known salts, will substantiate its identity. The mydriatic effect of atropin can not be relied upon as an absolute proof of its presence in a mixture of this kind, because cocain has mydriatic properties, but atropin is much less soluble in petroleum ether than is cocain, and while two or three shakings with the solvent will usually remove the cocain and leave nothing in the coca extract giving Vitali's test to go into the chloroform solution, most of the atropin will be left in solution and when removed by the chloroform may be identified by this reaction, by its characteristic microscopic salts, and by other tests. It should be remarked that the purple color was obtained only when working with coca extract. The alkaloids cocain and tropacocain purchased on the market did not respond to this reaction, and in fact a very pure petroleum ether residue was found to give but a slight purple color.

In the case of nux vomica and belladonna the difference in the physiological properties of the alkaloids taken in conjunction with their characteristic microscopic salts would be sufficient to remove any doubt as to whether one or both of these drugs were present. In this connection it is of interest to note that pure strychnin was found to give the Vitali reaction the same as the extract from nux vomica.

If a mixture of coca, belladonna, and nux vomica is suspected, the identification of the atropin presents greater difficulties; or if one finds both cocain and strychnin in a product, it is not always an easy matter to state absolutely whether atropin is or is not present. Careful examination of the microscopic appearance of the precipitates given with the different alkaloidal reagents is necessary, together with further substantiation by the physiological action of the chloroform residue on the pupil of the eye, after it has been fully established that the cocain has previously been removed by petroleum ether.

When experimenting with a mixture of this description the atropin may be separated by obtaining a concentrated solution of the alkaloids in dilute sulphuric acid and precipitating the strychnin and cocain by platinic chlorid. Atropin is not thrown down, and by filtering, adding ammonium hydroxid to the filtrate, and again filtering, the alkaloid may be obtained in a very pure condition, by shaking out with chloroform.

The writer was unable to confirm the reactions obtained by Beckmann with the alkaloids of veratrum veride.

The similarity in the reactions of yohimbin and strychnin are of interest, as the drug containing the former alkaloid has of late been exploited as a remedial agent for the same purposes for which strychnin has been used. It has been claimed to possess aphrodisiac properties, and might well be suspected in mixtures advertised for tonics and the like. When working with small quantities the two reactions of yohimbin were so much like those given by strychnin that one could not draw any conclusion as to which alkaloid was present. In the case of the oxidation reaction the play of colors was different, however, pure yohimbin giving first a purple, then a reddish shade changing to olive green, and if a large quantity was present the color was indigo blue, which soon changed to olive green. The reaction with ammonium vanadate was so nearly identical with that given by strychnin that no distinction could be drawn with any safety. With nitric acid and alcoholic potash a purple color appeared at once changing to a reddish shade, and with large quantities the purple changed to a chocolate which became black on warming.

Yohimbin forms very few salts which have any characteristic form under the microscope, while those given by strychnin are well defined and can be distinguished readily. Furthermore, yohimbin does not act the same as strychnin physiologically, neither does it possess the mydriatic properties of the belladonna alkaloids. It does not resemble the coca alkaloids further than by giving the purple color with alcoholic potash, for on evaporation of the solvent it is left as a solid mass, while the coca bases remain in a semiliquid condition for some time. It is further distinguished from cocain by not giving the ethyl benzoate test nor the characteristic crystalline picrate.

There is little danger of confusing strychnin with the principle of colocynth which gives the purple color with sulphuric acid and bichromate. With regard to the alkaloids of hydrastis, gelsemium, and sanguinaria, none of these give the Vitali reaction, and there are a number of well-defined reactions for these substances which have been fully described. (With ammonium vanadate berberin gives a red soon changing to plum color, and gelsemium a magenta changing to blue green.) In connection with the alkaloids of gelsemium, it might be noted that a mixture of the same with strychnin would, as far as the tests in question are concerned, give practically the same reaction as a mixture of atropin and strychnin, and as gelsemium has the property of dilating the pupil of the eye the physiological tests would not serve as final proof. Recourse must be had again to other distinctive reactions of gelsemium and to the appearance of the precipitates under the microscope. These facts are of importance because combinations of belladonna and nux vomica and of gelsemium and nux vomica are on the market.

The purple test given by the opium alkaloids could hardly be mistaken for that given by strychnin, as the color is very lasting in the former case. Some of the free alkaloids obtained from opium were treated with sulphuric acid and bichromate, and it was found that narcein and papaverin gave a purple color, while no similar reaction was obtained with thebain, narcotin, codein, or morphin.

THE SEPARATION AND IDENTIFICATION OF SMALL QUANTITIES OF COCAIN.

By H. C. Fuller.

It is well known that the separation and positive identification of cocain is very difficult even when it is present in large quantities, and when there is only a small amount present the separation is often claimed to be practically impossible. The work here recorded, however, demonstrates that these claims are unfounded.

Contrary to the statements appearing from time to time in the literature of the subject, cocain has no characteristic color reactions on which any reliance may be placed, and if the amount present is sufficient to respond to certain reactions which have been described it could be more positively identified by its melting point and by other well-known properties; with a small quantity the color reactions are not given and in the presence of other alkaloids the tests would have little or no significance.

This paper deals entirely with the separation of small quantities of cocain, and the procedures described have been evolved after considerable experimentation with various methods recommended in the literature for the separation of alkaloids and with others as yet unpublished. The writer has been called upon to examine a number of medicinal products and medicated beverages supposed to contain cocain, and especially in the latter the amount of alkaloidal substance has been comparatively small. It has been interesting to note that many chemists have reported that no cocain was found in some of these products, even after claiming to have made a careful examination of the same, while subsequent testing proved beyond a doubt that marked quantities were present. It was clearly evident that these workers were not acquainted with the procedure for effecting a proper separation.

Cocain and the other alkaloids of the coca leaf are readily hydrolyzed on heating; consequently it is advisable to keep the solutions at room temperature during the manipulation, and if it is necessary to heat at any stage of the process the solutions or residues should be cooled again as soon as possible. If the substance under examination is a solid it should be dissolved in water, if possible, or in normal sulphuric acid: if it contains much drug material and is not readily dissolved in water an extraction with alcohol containing a small quantity of ammonium hydroxid should be resorted to, water added to the mixture, and the bulk of the alcohol subsequently evaporated. Liquid products, such as sirups, need no preliminary treatment unless they are very thick or in the form of emulsion; in the former case they should be diluted to about the consistency of a 50 per cent sugar solution, and in the latter some method which will separate the gum and fatty material must be adopted.

Having obtained a clear solution of the substance in question the procedure is as follows:

Transfer the solution to a separator and add a slight excess of ammonium hydroxid. (If the product was originally alkaline it is best to acidify it and then add a slight excess of ammonia. If a precipitate is formed it should be separated by filtration and the filtrate used for analysis.) Shake out the solution with 50 cc of Prolius mixture (ether 4 parts, chloroform 1 part, and alcohol 1 part), and allow to stand; then collect the clear solvent in another separator and shake out the aqueous solution twice more with the Prolius mixture. Filter the combined solvent solutions through a creased paper into a beaker and evaporate the liquid over a steam bath, using a fan. Do not allow the residue to dry, but as soon as the last portions of alcohol are driven off remove the beaker from the steam. Add 25 cc of normal sulphuric acid in 10 cc portions, warm the mixture slightly and then filter into a separator after each portion of the dilute acid is added, and finally wash with a little water. Then shake out the solution five times with 15 cc portions of chloroform, preserving the chloroformic extracts in another separator. Wash the latter with 10 cc of distilled water, discard the chloroform, and add the wash water to the acid liquid which was shaken out with the chloroform. Then add 10 cc of petroleum ether (boiling at 40°–60° C.) and thoroughly shake the separator, separate the acid liquid, and discard the solvent. Add a slight excess of ammonium hydroxid, cool the mixture, and then shake out the solution with 15 cc of petroleum ether of the boiling point before specified and reserve the solvent solution in another separator. Shake twice again with the same quantity of solvent, then wash the combined petroleum ether solutions once with distilled water and filter into a small beaker, washing the separator and filter with 10 cc of petroleum ether. Evaporate the petroleum ether rapidly over a steam bath, using a fan, and if cocain was present in the original material it will be found in the residue.

By this method it is possible to obtain the cocain in a very pure condition; in fact, it will often crystallize in a few hours even though the amount present be very small. The identification tests are conducted as follows:

Dissolve the residue in petroleum ether and pour portions of the solvent into a beaker and a small evaporating dish, reserving the remainder for subsequent tests. After the solvent has evaporated dissolve the contents of the beaker in 5 cc of normal sulphuric acid, warming if necessary, and add potassium mercuric iodid test solution.

The formation of a precipitate indicates the presence of an alkaloid, but, of course, does not identify it as cocain; if no precipitate occurs further tests are unnecessary.

To identify the alkaloid treat the contents of the evaporating dish with 2 cc of concentrated nitric acid and evaporate the mixture to dryness over the steam bath. When there is no further odor of nitric acid remove the dish, cool, and add 5 to 10 drops of fifth-normal alcoholic potash solution, noting carefully the color and odor of the mixture while the dish is cool and then applying gentle heat and noting the odor again.

Minute traces of cocain will give off the odor of ethyl benzoate on treatment with nitric acid and alcoholic potash. The odor is very marked in its character and can be readily distinguished, though until one is familiar with it a parallel test should always be run, using pure cocain.

The color reaction is also of interest and will prove valuable in detecting the presence of other alkaloids. A purple color would indicate that atropin, strychnin, or yohimbin were present, though it is well known that a residue obtained by the above method from the coca leaf will in some instances also give a purple color. Tropacocain, benzoylecgonin, and aconitin will also give the ethyl benzoate test, but the possibility of the presence of the first two can be eliminated by a subsequent microscopic test, and from the fact that the benzoylecgonin is not removed from the aqueous solution to any great extent by petroleum ether. The latter fact is also true of aconitin, but the presence of this very poisonous alkaloid would be readily apparent when performing the physiological test.

The portion of the material which had been reserved should be tested physiologically. The petroleum ether is evaporated, a part of the cool residue is placed on the tongue and rubbed gently with the finger for about one minute, when, if cocain is present, a sensation of numbness gradually develops, the tongue and often the lips which have been touched by the finger will have a smooth, ivory-like feeling, and the effect will be apparent for some time. This is another test which should be performed in conjunction with one on a known sample, unless the analyst is perfectly familiar with the sensation.

This residue may also be used for a microscopic test by removing a small portion, placing it on a microscope slide and noting, under the lens, the character of the crystals found with a drop or two of gold chlorid solution. Cocain forms a definite crystalline compound with gold chlorid, and the product obtained should be compared with that given by a solution of the pure alkaloid.

With a residue obtained originally as described, these tests will substantiate the presence of cocain even though it occur in a minute quantity, and the four reactions can be applied to very small residues, although if it is desired the first mentioned with potassium-mercuric iodid may be omitted.

Another reaction for cocain which is very characteristic when considered in conjunction with the ethyl benzoate test is obtained by transferring some of the residue to a pressure flask, adding a few crystals of salicylic acid and about 15 cc of dilute hydrochloric acid, and heating the flask, stoppered, for about an hour and a half over the steam bath. On opening the flask the odor of wintergreen will be very marked if cocain was originally present. Tropacocain does not respond to this reaction, though it might indicate the presence of cinnamyl cocain and the truxillins; all of the latter, however, are precluded if the mixture gives the ethyl benzoate test.

THE DETERMINATION OF MOLYBDIC TRIOXID.

By B. Herstein.

The quantitative estimation of molybdenum and its compounds offers a number of difficulties. Of the titrimetric methods proposed for this purpose those based on the reduction with zinc and subsequent titration with permanganate solution[a] give persistently, though not consistently, too low results, while those based on iodometric methods[b] require great familiarity with the subject, scrupulous observations of empirical limitations, the concurrence of many favorable conditions, and the exclusion of all chance combinations which might interfere with the correctness of the results, which even then are not uniform. Of the gravimetric methods known, some are untrustworthy, as, for instance, precipitation as lead molybdate, while others require several repetitions of the operations to insure constant results. If a series of determinations is to be made, one must necessarily familiarize himself with the best conditions essential for the greatest degree of accuracy possible, but where molybdenum, or its compounds, is to be determined only occasionally, as is the case in most laboratories, a method is needed which, with ordinary skill and precaution, will give accurate results.

The method described in this paper is believed to meet this need. It involves the precipitation of molybdenum by means of thioacetic acid in a strongly acid solution, and the transformation of the precipitate so obtained into molybdic trioxid by mere ignition and weighing as such. Thioacetic acid under these conditions precipitates molybdenum quantitatively and within a short time, while hydrogen sulphid and similar reagents, as is well known, precipitate only partially and require several repetitions to complete the reaction. The precipitate formed by the thioacetic acid does not seem to be merely a combination of molybdenum with sulphur, as one would at first suppose, but apparently contains thioacetic acid within the molecule, since on ignition of the purified precipitate the penetrating odor of this acid is strong and unmistakable. The precipitate also seems to be less dense than the sulphid ordinarily obtained which greatly facilitates its transformation into molybdic acid when ignited.

Euler and Friedheim[c] in 1895 recommended that the molybdenum sulphid be transformed into molybdic trioxid and weighed as such. They, however, used paper filters which were incompletely ignited, and to separate the charred particles it was necessary to dissolve the molybdenum compound with ammonium hydroxid, evaporate, and ignite again in order to obtain the molybdic trioxid in a pure condition. By using a Gooch crucible these complications and potential sources of error are eliminated. The procedure is as follows:

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[a] V. d. Pfordten. Ber. d. chem. Ges., 1882, 15: 1927.

[b] Euler and Friedheim. Ber. d. chem. Ges., 1895 (2), 28: 2061; Gooch and Fairbanks. Amer. J. Sci., 1896 (4) 2: 156; and Gooch and Pulman. 1901 (4), 12: 449.

[c] Loc. cit.

The solution containing not more than 0.5 gram (preferably between 0.2 and 0.3 gram of molybdic trioxid) is placed in a pressure flask or a thick-walled Erlenmeyer flask of about 200 cc capacity and acidified with 5 cc of concentrated hydrochloric acid. A freshly prepared aqueous solution of thioacetic acid containing 0.75 cc of the pure acid for each 0.1 gram of molybdenum trioxid is then added, the flask securely stoppered, the mixture digested on a water bath for from forty to sixty minutes,[a] then cooled somewhat, filtered through a tared Gooch crucible covered with asbestos, and thoroughly washed with water containing about 2 per cent of hydrochloric acid. The use of too strong a vacuum is to be avoided. The particles which have crept up the sides of the crucible are washed down with a little alcohol, which at the same time replaces the water and facilitates the drying. The precipitate is dried at about 120° C. in the crucible, which is then set to half its depth in a close-fitting hole in a stout asbestos board and heated over a bunsen flame. The crucible is covered in such a way as to permit a little air to enter. The flame of the burner is to be kept very low at first until the thioacetic and sulphurous acids have disappeared, then it is gradually increased until the submerged part of the crucible shows dull redness and the heating is discontinued when the originally brown precipitate has assumed the yellow or slightly grayish color of molybdenum trioxid. The crucible is weighed after cooling in a desiccator. As a precaution the crucible may be reheated and reweighed.

Molybdic trioxid, as is well known, volatilizes in the open air at a bright red heat, but no loss need be apprehended if the directions given are followed. The protruding part of the crucible under these conditions remains sufficiently cool to condense any molybdic trioxid which might be vaporized. For the purpose of hastening the oxidation the cover may be removed after the volatile gases have ceased to come off, but it has been found that by allowing the oxidation to take its natural course a more uniform product is obtained.

The following figures, obtained on ammonium molybdate whose composition was carefully determined in various ways, show the availability of the method which, though not very rapid (requiring from 3 to 4 hours for a determination) has the advantage of giving reliable results:

Determinations of molybdic trioxid in known samples by proposed method.

Amount present.	Amount found.	Difference.	Amount present.	Amount found.	Difference.
Gram.	*Gram.*	*Gram.*	*Gram.*	*Gram.*	*Gram.*
0.08156	0.0815	−0.00006	0.2039	[b] 0.2005	−0.0034
.2039	.2040	+ .0001	.4078	.4072	− .0006
.2039	.2038	− .0001	.4078	.4063	− .0015
.2039	.2043	+ .0004	.4078	.4082	+ .0004
.2039	.2038	− .0001			

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[a] Thioacetic acid dissolves in water to the extent of about 6 per cent, but such a solution deteriorates very rapidly and after standing about one week is worthless. It is, therefore, necessary to work with a freshly prepared solution only.

[b] Owing to insufficient drying and the premature removal of the crucible cover some loss by decrepitation was sustained.

On account of the cost and rather unpleasant odor of thioacetic acid experiments were made substituting sodium thiosulphate for it, but although as much as 5 grams of the latter were used for 0.2 gram of molybdic trioxid and the heating under pressure was considerably prolonged the results were disappointing, the weight obtained in two instances being 0.0815 and 0.0977 gram instead of 0.2039 gram. Somewhat better, though by no means accurate, results were obtained when yellow ammonium sulphid was employed.

A METHOD FOR TESTING AMMONIUM SALTS.

By B. Herstein.

While titrating acid solutions containing both formaldehyde and ammonium salts serious discrepancies in the results were noticed, and in 1909 an extended investigation of the phenomena was begun. It was soon found that formaldehyde acts upon ammonium salts in the same way as it does upon ammonium hydroxid, namely, by forming hexamethylentetramin. It was also found that in the presence of a sufficient quantity of formaldehyde the ammonium ion is liberated from the acid with which it has been combined, and the latter thus becomes titratable with a standard solution of sodium or potassium hydroxid, using phenolphthalein as indicator.

In a paper entitled, "Trennung von Amin- und Säurefunction in Lösungen von Aminsäuren mittelst Formaldehyd," Schiff [a] reported the same reaction and later based thereon a method for determining formaldehyde.[b] He also stated that this reaction may be utilized inversely, that is, for determining ammonium salts by means of formaldehyde, but no details are given. A. Ronchèse[c] studied this reaction more carefully and recommends it as a method for determining ammonia. He uses, however, an unnecessarily large excess of formaldehyde which tends seriously to impair the results. In view of the fact that ammonium salts play a very important part in every chemical laboratory, an easy method for their quantitative determination is very desirable. The best method hitherto available was to add an excess of standard fixed alkali, heat until all the ammonia is expelled, and then titrate back the excess of alkali used by means of a standardized acid solution. This method has disadvantages, chief among which is the time required to drive off all ammonia. The following method requires a minimum amount of time and yet gives satisfactory results:

A weighed quantity, about 1 gram, of any ammonium salt is dissolved in approximately 50 cc of water, 3 or 4 cc of a perfectly neutral 40 per cent solution of formaldehyde, and a few drops of phenolphthalein are added, and titrated with a standard solution of a fixed alkali until the pink color remains permanent after the solution has been brought to boiling. The previous neutralization of formaldehyde is necessary, because it almost invariably contains some formic acid formed by spontaneous oxidation; of the many indicators tried phenolphthalein alone gave satisfactory results.

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[a] Liebig's Annalen der chemie, 1901 : 319 : 76.

[b] Chem. Ztg., 1903, 27 (1) : 13

[c] J. chim. pharm., 1907 (6) 25 : 611.

It can readily be seen that inasmuch as the formaldehyde eliminates the ammonium ion as a factor so far as the titration is concerned, the result obtained will always express the total acid present, either free or combined with ammonia. In the absence of organic acids an additional titration without formaldehyde, using methyl orange as indicator, will show whether, and to what extent, either the acid or the ammonia is in excess.

It is hardly necessary to point out that this method is applicable not only to relatively pure ammonium salts, but also to many mixtures containing such, provided they do not contain salts of metals precipitable by alkaline hydroxids. The method was tested on the following ammonium salts with satisfactory results: Ammonium chlorid, bromid, iodid, fluorid, sulphate, acetate, oxalate, citrate, thiocyanate, and molybdate.

The method was also tested by using known quantities of standardized acids, hydrochloric, sulphuric, nitric, and oxalic, neutralizing these either partly or completely, with an excess of ammonium hydroxid and then titrating as previously described in the presence of sufficient formaldehyde and a few drops of phenolphthalein with standard sodium hydroxid.

In the well-founded opinion of Schiff, formaldehyde in reacting upon ammonium salts forms hexamethylentetramin according to the following equation:

$$4NH_4Cl + 6CH_2O = 4HCl + N(CH_2N + CH_2)_3 + 6\ H_2O$$

During the reaction, especially in the case of weaker acids, much heat is evolved, and the avidity of the formaldehyde for the ammonium ion is so great that an aqueous solution of ammonium acetate, for instance (which ordinarily on heating gives off considerable ammonia) on boiling with formaldehyde in excess loses nearly all of its acetic acid, hexamethylentetramin remaining.

CHARACTER OF SAMPLES OF BEESWAX SUBMITTED WITH BIDS.

By L. F. Kebler and F. M. Boyles

INTRODUCTION.

On account of the great variation in price, the uncertainty as to the quality of the samples, and the fact that if an award was made on an adulterated sample it would constitute the basis of purchase for a year, the departmental general supply committee referred 26 samples of beeswax, submitted with bids, to the Bureau of Chemistry for examination. The results given in the accompanying table clearly show that the price is not a reliable index as to quality. In fact, the sample of white beeswax for which the highest price was asked, namely, 60 cents a pound, was adulterated, not only with paraffin but with stearic acid as well. The prices quoted for yellow beeswax varied over 100 per cent, from 18 to 38 cents per pound, and for white beeswax the range is still greater, namely, 24 to 60 cents, yet some of the high-priced as well as some of the low-priced products were adulterated. Of the 26 samples examined, 53.8 per cent were adulterated. The more common adulterants were paraffin and ceresin; artificial coloring was detected in only one case. The examinations were made by the following methods:

METHODS OF ANALYSIS.

Specific gravity.—Drop the melted wax by means of a stirring rod onto a moist, cool, smooth porcelain surface in such manner as to obtain globules about one-eighth of an inch in diameter; allow to cool for from 2 to 24 hours, then place in a suitable glass cylinder of about 200 cc capacity and add a cooled mixture of alcohol and water having about the specific gravity of beeswax. If the globules sink, add water; if they rise to the surface, add alcohol. The water or alcohol is added with repeated agitation until the globules float indifferently in the mixture at the desired temperature. The specific gravity of the mixture is taken at this point with a pyknometer.

Melting temperature.—Thinly coat the bulb of a thermometer with wax and allow it to stand 24 hours. Place the bulb of the thermometer in a large test tube, holding it in place by a stopper grooved on the sides so as to allow free access of air. Immerse the test tube containing the thermometer in a beaker of water and raise the temperature very gradually (1 degree in from two to three minutes). The temperature at which a transparent drop forms on the end of the thermometer bulb is taken as the melting point.

Acid, ether, and saponification numbers.—Place 5 grams of the wax in a 200 cc Erlenmeyer flask with about 25 cc neutral alcohol and heat on the water bath until the mixture is entirely melted. Then add 1 cc of phenolphthalein solution and titrate the free acid quickly with seminormal alcoholic potash. Add 50 cc more of the seminormal alcoholic potash and heat the mixture for at least three hours over a direct flame with a reflux condenser. The excess of potassium hydroxid is determined with seminormal hydrochloric acid. A blank with the alcoholic potash should be run.

Stearic acid.—Boil 1 gram of the wax for several minutes with 15 cc of 80 per cent alcohol, allow to cool to between 18° and 20° C., filter into a 200 cc cylinder, and dilute filtrate with water to about 200 cc. If stearic acid is present, it separates into flakes and collects at the surface. The test is sensitive to 1 per cent. If from 7 to 8 per cent are present, a thick, creamy mixture results.

Resin.—Method I: Keep at the boiling point for about one minute a mixture of 5 grams of the wax and 20 to 25 cc of nitric acid (specific gravity, 1.32 to 1.33), dilute with an equal volume of water and render slightly alkaline with ammonium hydroxid. The solution decanted from the separated wax in the absence of resin is of a yellowish color, while its presence produces a more or less intense reddish-brown coloration.

Method II: Heat 1 gram of wax for a few minutes with 25 cc of 50 per cent alcohol (alcohol of this strength will not extract stearic acid if present); cool and filter; evaporate the filtrate to dryness on a water bath and add 5 cc acetic anhydrid. Heat to boiling, cool, and then carefully allow sulphuric acid (specific gravity, 1.53), to flow into the solution. The presence of the minutest quantity of resin will develop a fine violet (fugitive) color.

Detection and estimation of paraffin.—The following method is the only one by which paraffin can be detected with certainty: Melt from 2 to 5 grams of the wax in a porcelain dish, then add an equal weight of finely powdered potassium hydroxid, continue heating for a few moments with thorough stirring. Cool and powder the hard mass, and mix the resulting powder with three times as much potash lime as wax used, then introduce this mixture into a thick-walled tube, immerse in an oil bath, and heat to about 250° C. for from three to four hours. After cooling, finally powder the tube with its contents, place the mass in a Soxhlet apparatus, and extract with petroleum ether for several hours. The petroleum ether is evaporated and the residue dried at 110° C. and weighed. By this treatment the esters are converted into alcohols, and these alcohols on heating with potash lime are in turn converted into their respective acids, while the hydrocarbons present are not affected and are extracted with petroleum ether. Pure beeswax contains naturally from 12.5 to 14 per cent of hydrocarbons, and any adulteration with paraffin or allied bodies will increase this percentage.

The Henriques[a] *cold saponification method.*—This method was found very satisfactory. The wax is dissolved in high-boiling-point (100° to 150° C.) petroleum ether, alcoholic potassium hydroxid is added in excess, and the whole, after being warmed on the water bath, is allowed to stand twenty-four hours, when the excess of potassium hydroxid is titrated with seminormal hydrochloric acid, phenolphthalein being used as indicator.

The methods of the United States Pharmacopœia for testing beeswax are on the whole unsatisfactory. The carbonization of wax with sulphuric acid for detecting paraffin is unwieldy, disagreeable, and unreliable. Small amounts of paraffin can not be detected with any degree of certainty by this method. Again, the method for saponifying beeswax by heating on the water bath for one-half hour with alcoholic potassium hydroxid is uncertain.

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[a] Zts. angew. Chem., 1895, 81: 721; (Abst.) Analyst, 1896, 21: 192.

TABULATED RESULTS OF EXAMINATION.

In the following table are given the results of the tests made to determine the quality of the samples of beeswax, together with the prices quoted for the same:

Results of the examination of 26 samples of commercial beeswax submitted with bids.

Number and color.	Price quoted.	Melting point.	Specific gravity at 25° C.	Acid number.	Ether number.	Saponification number.	Ratio of acid number to ether number.	Adulterants.
Yellow:	*Cents.*	*°C.*						
1....	35.67	63.0	0.9588	20.40	69.40	89.80	3.40	Absent.
2....	34.75	63.5	.9562	18.94	72.74	91.68	3.84	Do.
3....	35.00	56.0	.9237	7.09	26.98	34.07	3.80	Paraffin.
4....	33.25	63.0	.9623	20.40	71.46	91.86	3.50	Absent.
5....	31.50	58.0	.9198	5.55	20.90	26.45	3.76	Paraffin and artificial color.
6....	30.00	55.0	.9286	28.21	18.16	46.37	.64	Paraffin and stearic acid.
7....	32.00	70.0	.9832	34.20	86.44	120.64	2.52	Resin.
8....	25.00	57.0	.9284	7.25	31.60	38.85	4.03	Paraffin.
9....	18.00	54.0	.9127	3.38	39.30	42.68	11.60	Do.
10....	34.24	63.0	.9564	17.65	73.18	90.83	4.14	Absent.
11....	34.24	63.0	.9599	18.21	74.91	93.12	4.11	Do.
12....	43.00	66.5	.9389	10.47	44.30	54.77	4.23	Ceresin.
13....	21.00	56.0	.9144	4.83	15.71	19.54	3.24	Paraffin.
14....	34.24	63.0	.9595	17.65	73.60	91.25	4.16	Absent.
15....	34.20	63.0	.9529	18.53	76.53	95.06	4.13	Do.
16....	32.00	63.5	.9609	18.37	73.30	91.67	3.99	Do.
17....	34.50	62.5	.9529	17.33	73.30	90.63	4.22	Do.
18....	28.00	52.5	.9126	3.62	11.42	15.04	3.15	Paraffin.
19....	38.00	64.0	.9483	12.48	62.04	74.52	4.97	Ceresin.
20....	24.00	52.5	.9132	4.03	12.43	16.46	3.08	Paraffin.
21....	45.00	62.5	.9583	18.00	72.74	90.74	4.04	Absent.
White:								
22....	45.00	63.0	.9603	17.83	74.52	92.35	4.17	Do.
23....	48.00	62.0	.9526	15.44	61.40	76.84	3.98	Ceresin.
24....	60.00	55.0	.9295	55.22	11.42	66.64	.21	Paraffin and stearic acid.
25....	24.00	56.0	.9124	4.43	12.43	16.86	2.80	Paraffin.
26....	46.00	63.0	.9632	20.90	73.02	93.92	3.49	Absent.

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BUREAU OF CHEMISTRY—BULLETIN No. 151.

H. W. WILEY, Chief of Bureau.

THE CANNING OF FOODS;

A DESCRIPTION OF THE METHODS FOLLOWED IN COMMERCIAL CANNING.

BY

A. W. BITTING,
Food Technologist, Bureau of Chemistry.

WASHINGTON:
GOVERNMENT PRINTING OFFICE.
1912.

LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Chemistry,
Washington, D. C., December 6, 1911.

Sir: I have the honor to submit for your approval a bulletin on the canning of foods, prepared by A. W. Bitting, food technologist of this bureau, who has conducted quite extensive investigations along these lines, both in the field and in the laboratory. The preserving of foods by heat in sealed containers has become a most important industry, not only from a commercial standpoint, but also from that of good living. The principles involved in the work are not particularly new, but some of them are not generally understood. Many of those engaged in the work of canning follow certain steps according to a prescribed formula rather than from a knowledge of why they are necessary. The consumer often accepts the contents of a can in lieu of the fresh article as a matter of necessity but with misgiving as to its wholesomeness.

The object of this bulletin is to give, in a rather popular form, a description of the conditions in the better type of factories and the methods followed, so as to indicate to manufacturers what is expected in a modern plant; to give to teachers of domestic science more nearly accurate information upon this line of work than is now available; and to inform the consumer what goes into a can and what he may reasonably expect. The description is general and there are many variations due to local conditions which it is not possible to detail. Some of the trade practices are not wholly approved by this bureau and their presentation here does not mean an indorsement. There are changes going on, particularly with regard to grading and labeling, which in time will make it possible for the purchaser to know just what he is buying. The purely technical investigations on the composition of the products, the physical and chemical changes which they undergo in canning, the effect of the container upon the food, the bacteriology of spoilage, etc., have been withheld for future consideration.

I recommend the publication of this manuscript as Bulletin No. 151 of the Bureau of Chemistry.

Respectfully,

R. E. Doolittle,
Acting Chief.

Hon. James Wilson,
Secretary of Agriculture.

CONTENTS.

Page.
Historical note 7
Early theories on preservation by canning 10
Sterilization 12
  Historical note 12
  Modern methods of sterilization 13
  Determination of temperature and time of processing 15
Modern factory equipment and methods 16
  Sanitation 16
  Methods and processes 20
    Raw materials 20
    Grading 20
    Washing 21
    Preparation and blanching 21
    Washing and filling the cans 22
    Exhausting 22
    Capping and testing for leaks 22
    Processing and cooling 23
Containers 24
The label 26
Use of the term "canned" 28
Spoilage 28
Effect of heat and cold 30
Home canning 31
Cost of canned foods compared with fresh 32
Extent of the canning industry in the United States 33
Detailed consideration of the various products 36
  Fruits 36
    General discussion 36
    Apples 37
    Apricots 38
    Blackberries 38
    Cherries 38
    Gooseberries 39
    Grapes 39
    Peaches 39
    Pears 41
    Plums 41
    Raspberries 41
    Strawberries 42
  Vegetables 43
    Asparagus 43
    Beans, green 44
    Beans, Lima 45
    Beans, wax 46

Detailed consideration of the various products—Continued.

Vegetables—Continued.

Page.
Beets.. 46
Corn, sweet.. 47
Peas... 50
Pumpkin.. 54
Rhubarb.. 55
Squash (*see under* "Pumpkin")............................... 55
Succotash.. 56
Sweet potatoes... 56
Tomatoes... 57
Marine products.. 61
Crabs.. 62
Oysters.. 62
Salmon... 66
Sardines... 68
Shrimp... 69
Milk... 71
Specialties and soups.. 72
Beans, baked... 72
Hominy... 74
Sauerkraut... 75
Soups.. 76

THE CANNING OF FOODS.

A DESCRIPTION OF THE METHODS FOLLOWED IN COMMERCIAL CANNING.

HISTORICAL NOTE.

Canning is the art of preserving a food product in a hermetically sealed container, the preservation being accomplished through sterilization by means of heat. In its highest sense the object is to retain the food in as nearly a fresh condition as possible as to appearance, palatability, and nutritive quality or in the condition in which it is usually consumed. It affords the means of having wholesome succulent vegetables or other products at all times and in places where otherwise the cost or the labor of preparation would be prohibitive. The art was evidently of slow development and the result of various dissociated experiments. The real foundation was laid by Spallanzani, who in 1765 made experiments which disproved the then popular theory of spontaneous generation. These consisted in placing various nutritive liquids in tubes, sealing them, and then boiling in a water bath for an hour. He showed that the liquids remained unchanged as long as the seal was unbroken and free from external air. He therefore concluded that the "eggs" which cause spoilage are somehow carried in the air. This was canning on a very small scale. The experiments also demonstrated that there was a difference in the effect of moist and of dry heat; that whereas life was destroyed by water at a temperature of 45° C., in the dry state 80° C. was necessary.

The first practical application of this discovery was made by Scheele, a Swedish chemist and apothecary, who preserved vinegar by boiling it in jars or bottles and sealing it at once. This was in 1782, and at that time the keeping of good vinegar for extracts and other pharmaceutical processes was of much greater importance than can be realized now. There is no record, however, that Scheele carried his work further than the preservation of the pharmaceutical product.

In 1795 Nicholas Appert, a Frenchman of exceptional training in experimental work and of large practical experience in confectioneries, kitchens, breweries, and distilleries, began work on food preservation. His work was stimulated by offers of reward on the part of the Government for better methods of preparing foods for the army and navy. According to Appert's own story he was unsuccessful until 1804. At that time he learned the secret of preserving by heat in a sealed container, then continued his experiments until 1810, when he published his methods in detail. According to the records he succeeded with many products and produced results which it would be difficult to improve upon at this time. The essential points in the method described by him are that the food to be preserved is placed in glass bottles with water and corked carefully, after which the bottle is placed in a water bath and heated for a period dependent upon the nature of the food used. At the end of the heating period the bottles are removed from the bath and cooled. By this method the temperature attained was from 190° to 200° F., the maximum being 212° F. He says, "I chose glass as the matter most impenetrable by air and have not ventured to experiment with vessels of any other substance." Appert's publication won for him the prize of 12,000 francs offered by the French Government and general recognition as the founder of the art of canning.

In 1807 a Mr. Saddington presented a paper before the English Society of Arts, entitled "A method of preserving fruits without sugar for house or sea stores." Mr. Saddington did not claim the discovery of the method, and being a traveler it is supposed that he learned the general facts abroad, though he may have made some modifications. His method was essentially as follows: The fruit was placed in bottles and loosely corked. The bottles were placed in cold water up to the neck, heat applied gradually until 75° C. was reached, and then kept at that temperature for an hour. After this, the bottles were filled with boiling water, corked lightly, and laid on their sides to swell the cork. Later the corks were cemented.

Peter Durand, early recognizing the possibilities of preserved foods, took out an English patent in 1810, covering both the preserving of "animal, vegetable, and other perishable foods," and also the container, covering "vessels made of glass, pottery, tin, or any metal, or fit materials." The essential feature of the preservation was that the food be heated and hermetically sealed in the container. This is the first record of using the tin can as a container; therefore its invention is attributed to Mr. Durand. It is known, however, that even previous to 1800 the Dutch put up fish in tin, though their methods were not those now generally recognized for canning. The fish, on being caught, were dressed at once, boiled for a short time in salt brine, and smoked for two days, after which they were put in the cans, covered with hot butter or olive oil, and sealed. The tin can was practically coincident with the art of canning.

The first improvement in the tin can, adapting it especially to canning purposes, was made in 1823 by Pierre Antoine Angilbert, a Frenchman. His improvement consisted in first putting the fruit and water in the can and covering it with a lid having a hole in it. The cans were set in a water bath and boiled for some time, after which the hole was closed with a drop of solder. The first patent on a tin can to be issued in this country was in favor of Mr. Thomas Kensett in 1825.

All the early tin cans were made by what was known as the plumb joint; that is, the edges along the sides were butted together and soldered, as were also the two ends. The entire work was done with a pair of scissors and a soldering iron. Only a few cans could be made in a day, 100 being considered a very large number for one workman. It was not until 1847 that Mr. Allen Taylor invented the stamped can with the extension edges. In 1849 the pressed top was added as an improvement.

From the beginning, bottles were too costly and broke too easily to be used for the cheaper articles of food. The earthenware jars were heavy and not sufficiently well glazed. The tin can lent itself to commercial purposes best, but it was expensive and the evolution of its manufacture was slow. At present it is manufactured by automatic machinery at low cost and in enormous quantities.

At first the temperature employed in canning was from 190° to 212° F., but this was found to be insufficient for many products. A higher temperature was secured by adding salt to the water in the bath, and later this was gradually supplanted by calcium chlorid. With the latter it was possible to secure a temperature of 240° F., thus shortening the period of cooking and also making it possible to process some products which could not be sterilized by boiling alone. It was not until 1874 that Mr. A. R. Shriver, of Baltimore, invented the closed-process kettle for cooking with superheated water. About the same time, Mr. John Fisher, of the same city, patented a kettle in which live steam could be used. These same methods, boiling in water in the open, in a bath of calcium chlorid (now being superseded by oil), in superheated water, and with steam in closed kettles, are still in common use, the only difference being in certain modifications for doing the work easily. The most recent addition to the equipment for heating is an agitator, which has the effect of stirring the contents inside the cans so that all parts are heated uniformly and rapidly, and is advantageous for certain products.

Shortly after Appert's method had been published and the tin can found to be suitable as a container, small canneries were established in Aberdeen, Scotland, in Sligo, Ireland, and in European fishing ports. In 1845 the canning of sardines was begun in France; in fact, the early products were nearly all sea foods.

The first canner in this country was Ezra Daggett, who learned the trade abroad. He and Thomas Kensett packed a few salmon, lobsters, and oysters in New York in 1819. The next year William Underwood and Charles Mitchell began operations and a factory was opened in Baltimore. In 1839 Isaac Winslow began experimenting with the canning of corn in Portland, Me. His early efforts were mostly failures, but he had a persistence worthy of any cause, and by continuous work he felt warranted in 1852 in asking for a patent. So skeptical was the Patent Office that letters were not granted until 1862. In 1841 the first real fish cannery was established at Eastport, Me., the product being lobsters and mackerel, and by 1860 there were a number of canneries on the coast, handling both fish and vegetables. The first cannery in the Central States seems to have been established in the early sixties in Cincinnati, closely followed by one at Indianapolis. Canning was begun at San Francisco in 1856 and in the Alaskan waters in 1878.

The Civil War gave the first great impetus to canning in this country. That event showed the enormous advantage of having canned foods and emphasized their general superiority over the dried foods in palatability. The more recent extension of the industry has been due largely to a better knowledge of the wholesome character of canned products and the economy in their use.

EARLY THEORIES ON PRESERVATION BY CANNING.

From the beginning there were numerous theories explaining the preservation of foods by canning. The first was that of the exclusion of outside air. This theory was recognized in part by Appert in his description of the preservative process.

> It is obvious that this new method of preserving animal and vegetable substances proceeds from the simple principle of applying heat in a due degree to the several substances, after having deprived them as much as possible of all contact with the external air. It might, on the first view of the subject, be thought that a substance, either raw or previously acted upon by fire, and afterwards put in hot bottles, and they completely corked, [would] be preserved equally well with the application of heat in the water bath. This would be in error, for all the trials I have made convince me that the absolute privation of the external air (the internal air being rendered of no effect by the action of the heat) and the application of heat by means of a water bath, are both indispensable to the complete preservation of alimentary substances.

Appert did not know what was in the air to cause spoilage, but did recognize that it was the external and not the internal air. At this time some foods and wines were being preserved by excluding the air, the method being to cover the surface of the food or wine with hot oil. The experimenters, following Appert, laid special stress on excluding air, and when tin cans were first used care was taken to heat the contents well before sealing; later the cans were heated, then vented, and again heated. Sometimes a second venting was given. The belief was general that every trace of air must be removed. This theory was held until recently, particularly for meat and fish products, corn, and others difficult to process, as there was no recognition of the more resistive varieties of bacteria found on some substances. The theory of exclusion of air has had followers in the practical operations up to the present time.

The next theory advanced was that it was the vacuum which protected the food substances. This was believed by the workmen almost from the beginning, as they found that heating and then sealing the can resulted in more or less of a vacuum. This theory was closely associated with that of excluding the air, and those believing in it developed mechanical methods for producing the vacuum; these, however, are not of as much practical importance in canning as expected. The cold vacuum is useful in preserving meat and fish products which have been cured by salting, drying, or smoking, for fruits which have been partly cured by drying, sugaring, etc., and for jams, jellies, preserves, etc. The vacuum aids in preserving where the organisms causing spoilage require oxygen for growth.

In factory practice more or less of a vacuum is secured by passing the cans through an exhaust box which will heat them to 180° or more, or by adding hot brine or sirup before the can is sealed. To some products, particularly meat and fish, heat is applied, and the can vented to drive out the air; essentially the same result is accomplished in home canning in that the caps are not tightened until the contents are thoroughly heated. A recent procedure is to cap the cans and seal the vent in a strong vacuum chamber, instead of heating and later venting. The tin can with a good vacuum always shows the ends drawn in when cooled. Cans packed and sealed cold will have the ends flush or upon becoming warm they may spring out, producing so-called "flippers" or "springers," and while not spoiled, they have the appearance of swells and may not be merchantable. While the rôle of the vacuum as an aid in preserving is not to be underestimated, it has not the importance in canning that was attributed to it.

The theory of the arrest of chemical activity was early advanced as being effected by hermetic closure. The French Government appointed Gay-Lussac to investigate the cause of the preservation as introduced by Appert. Gay-Lussac reported that decomposition was a series of oxidation changes in a substance, and that driving out the air and preventing its ingress prevented these changes from taking place. This explanation was satisfactory until microorganisms were discovered and their relation to spoilage proved.

STERILIZATION.

HISTORICAL NOTE.

The present status of our knowledge of canning is based on the modern science of bacteriology. The invention of the compound microscope and its many improvements has revealed the presence of minute vegetable organisms in the air, in water, and on everything with which we come in contact. These organisms are bacteria, yeasts, and molds, and are present either in their vegetative or spore state, being the "eggs" which Spallanzani thought were in the air.

Bacteria and germs were first seen in 1683 by Leeuwenhoek, who constructed a crude compound microscope. He knew nothing of their character nor of their relation to higher organisms, but as many of them were motile he regarded them as animals, and from their habitat named them "infusoria." Almost nothing further was discovered until 1786, when O. F. Müller described some of the structural characters. Another lapse occurred until 1838, when Ehrenberg published a work upon the so-called infusoria, in which he added to and systematized the information to date, some of his names being still used. Dujardin, Perty, Cohn, and Nägeli added much to the knowledge of the structure and life habits of micro-organisms, but none of these associated the organisms with any economic problem.

Pasteur, about 1860, was the first to discover that these organisms were the cause of the changes known as fermentation; also the cause of the supposed spontaneous generation. He recognized that they had a definite relationship to economic activities and later he applied the knowledge gained in the laboratory in a practical way to many industries. He discovered that by heating certain food products to a certain degree and for a certain period the time of keeping might be prolonged. The principle had a practical application to many substances on which boiling would have an objectionable effect and is taken advantage of on a large scale in wine and beer making and in dairying. He found further that by heating and cooling at intervals, as on successive days, sterilization might be accomplished; also that the organisms on different substances had varying resistant powers to heat and that in intermittent heating different temperatures must be employed, according to the predominating organism. All of the fundamental principles that Pasteur discovered have an important relation to canning.

In 1876 Dr. Robert Koch discovered the relation of specific germs to diseases, and a few years later elaborated methods for separating germs into pure cultures. Since that time progress has been rapid and the relation of various organisms to the different industries has become fully established.

The first direct application of bacteriological methods to spoilage problems in canning in this country seems to have been made by H. L. Russell, of Wisconsin, in 1895.[1] He investigated the cause of swells in peas and found it to be due to bacteria as a result of insufficient processing. The Wisconsin packers were processing the peas at 230° F. for from 10 to 11 minutes, and were having heavy losses. He advised, as a result of his experiments, that the temperature be raised to 242° F. and maintained for 15 minutes, which caused a cessation of the trouble.

In 1896 Prescott and Underwood[2] began the study of swelled and soured corn, and later turned their attention to soured peas. Their first results were published in 1898. In that year they gave the first of a series of addresses before the National Canners' Association, which had a most marked effect in establishing canning on a scientific basis. They identified the bacteria causing spoilage in corn and peas, determined the thermal death points, the degree of heat attained in the center of cans, and the time required to attain it.

In 1897 the Canadian Government caused work to be done on spoilage in canned lobster, or black lobster, as it was called. It was determined that it was due to bacteria.

In 1903 Harding and Nicholson,[3] of the New York Experiment Station, studied the swelling of peas; they gave additional descriptions of the organisms, and, as a result of their experiments, recommended that the process be changed to 240° F. for 30 minutes.

Since 1900 there have been numerous contributions on the organisms causing spoilage. These usually deal with one particular product or some special phase of bacterial activity. There has been no systematic effort made to classify the organisms, to determine their relation to different products, the kind of spoilage produced, nor the exact methods of dealing with each. Several large packers employ experts to work out their own problems, but hold the results secret. It is evident that in the preparation of food there is a rich field for scientific research, with great possibilities for valuable and practical results.

MODERN METHODS OF STERILIZATION.

Sterilization may be accomplished by heat below, at, or above the boiling temperature, depending upon the length of time the heat is applied and the number of applications made. It is not practicable to sterilize all foods in the same way because of injury to quality or prohibitive expense. Sterilizing below the boiling point is feasible only for a few products, principally fruits, and then is advisable only when it is desired to preserve a very fine appearance. This may be accomplished above 180° F. by maintaining the temperature for a longer time than when boiling, or by repeating the operation on two or more successive days. The object is to prevent breaking the tissue and loss of juices from the fruits by excessive heat. This method of sterilization has been applied experimentally and in private canning with gratifying results, but it involves so much time and labor that it is not used commercially except in a limited way. Sufficient work has not been done to say definitely what products can best be treated in this way nor what temperatures are best suited for different foods. It has been used chiefly with goods in glass, though equally satisfactory results are obtained with foods in tin.

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[1] Wisconsin Agr. Exper. Sta. Report, 1895, pp. 227–231.

[2] Technological Quarterly, 1898, pp. 6–11.

[3] New York Agr. Exper. Sta., Geneva, 1903, Bul. 249.

Cooking at boiling temperature is practiced with nearly all fruits, as the germs present are easily destroyed. Most of the fruits are processed for from 12 to 25 minutes. The tomato is the most important vegetable processed at boiling temperature, which is usually maintained for 50 minutes.

Cooking at a temperature above the boiling point is necessary or advantageous for most vegetables, fish, milk, and meats. It is accomplished in retorts where steam is admitted under pressure, in retorts where water can be superheated, on the open calcium chlorid or oil bath.

Among the vegetables requiring a high temperature in processing are corn, peas, beans, both green and dry, pumpkin, beets, and sweet potatoes. Corn is one of the difficult products to can, requiring a temperature of from 245° to 250° F. for from 75 to 80 minutes, depending to a considerable extent upon how dry it is packed. If very dry, the heat will penetrate to the center of the can very slowly, the actual time required to raise the center to the temperature of the bath being from 55 to 65 minutes. In a can of peas this is accomplished in 6 or 7 minutes, the difference being due to the fact that heat currents are set up in the liquid portion of the peas while they are absent in the corn. The necessity for a high temperature is therefore dependent upon the ease with which the heat can penetrate the product, as well as the resistance of the organisms. Some products which were formerly processed by boiling for a long time are now given a higher temperature for a few minutes, as the product has a much better appearance when it is not overcooked.

Meat products, as a rule, contain highly resistant organisms, besides which the majority of these foods are of such a consistency that the heat penetrates them very slowly. As a class they require the heaviest process. Milk also contains very resistive germs, but being liquid it heats rapidly; in order to keep it smooth and prevent the portion in contact with the tin from scorching, the cans are turned or agitated almost continuously during the cooking.

DETERMINATION OF TEMPERATURE AND TIME OF PROCESSING.

In sterilizing, the heat must be applied equally to all cans, and it is therefore necessary to deliver steam at the bottom of the kettle, whether open or in a retort, to insure a circulation of the heat. In retorts, whether steam or hot water is used, there must always be a vent open to give off steam in order to hold the heat uniform at all points. The thermometer is the all-important tester, for if it does not show the proper degree of temperature, spoilage will follow. To test the uniformity of temperature in a retort, self-registering thermometers are sealed in a number of cans when placed in the crates, the cans are marked, and when the cooking is completed the thermometers are examined and compared, so that the heat may be adjusted until all give like readings. In a similar manner the time required for the heat to reach the center of the can is obtained, experimental lots being run for varying periods, and the temperature noted. The calcium chlorid or oil bath acts in the same way as the open water bath.

The writer employs two methods of determining the temperature in the center of a can and the rate of penetration. First, a thermometer is placed in a packing joint which is soldered into the can so that the bulb will just reach the center. By placing a collar an inch above the gasket the can may be submerged in oil and heat applied until a certain temperature is reached. The length of time necessary for the thermometer inside the can to reach the same point as that on the outside, or within from 2° to 5° of it, as experience demonstrates may be sufficient, must be allowed in the retort and the heating then continued for such an interval as may be found necessary for sterilization. For example, if the spores of certain organisms are killed at 230° F. in 12 minutes, and it should take 20 minutes to cause the content of the can to become heated, it would require 32 minutes as a minimum for processing, and as a margin of safety the recommendation would be for a longer time, probably for 40 minutes.

The second method of determining temperature in different parts of the retort and in the center of cans is to seal a thermocouple in the can and connect it with a recording apparatus. Thus a time and temperature curve is obtained directly. One of the important points learned from the latter apparatus was the effect of stirring or agitating the contents of cans which ordinarily required long cooking. A can of corn in a retort requiring 65 minutes to reach 245° F. requires only 30 minutes when rolled back and forth. The effect of the agitation was a shorter cooking, a brighter color of the corn, and a bright can on the inside. The principle is good, but some mechanical difficulties in successful operation have yet to be overcome.

The varying temperatures and methods used in canning produce a certain amount of strain on the cans. These strains also vary with the temperature at which the tipping is done. The contents of a can expand with heat and contract upon cooling. If a can of corn is tipped at 190° and placed in the retort to process, it is at once subjected to an external pressure of from 10 to 13 pounds per square inch, and the expansion of the contents will increase until the internal pressure equals or slightly exceeds the external, or it rises to from 12 to 14 pounds per square inch. During a part of the cooking process the strains become equalized, or nearly so, but when the cooking is stopped and the retort is thrown open there will be an outward strain equal to the internal expansion, and this will gradually decrease until the temperature falls to that at which the tipping is done. When the temperature drops to ordinary room temperature, instead of there being an internal pressure it is from without and at 70° will amount to 7 pounds. This accounts for the ends of the cans being drawn in. The can has therefore undergone a strain, first of pressure from without on being placed in the retort, then from expansion from within when the retort is opened, making a total strain of 25 pounds per square inch. The smaller cans, such as No. 2 or below, are not much affected by these strains, but in the larger sizes leaks are likely to occur. The more suddenly these strains are applied, as by the wide opening and closing of the steam valve, the greater the danger, and the turning on of a large volume of cold water results in buckling. This principle holds good for all products, the variation from these figures depending upon the temperature used.

MODERN FACTORY EQUIPMENT AND METHODS.

SANITATION.

A modern cannery is no longer the rough, crude shed that once was thought to be sufficient for this purpose. First of all the location must be sanitary, away from manufacturing processes which of themselves are objectionable, such as soap making, tanning, rendering fats, etc., or any other processes which may give rise to noxious odors or be productive of organisms of decomposition. The yards and drives about the factory should be cleaned daily, and in summer dust should be prevented by frequent sprinkling or by the application of crude or specially prepared oil to the drives. The application of oil is especially to be recommended where there is much hauling and there is no pavement, or the factory is to be run for a short season only, as in the case of tomatoes. A single application made a couple of weeks before the season opens will suffice for several weeks; by putting the oil on early it will become incorporated in the earth and not be tracked into the factory to any great extent. The drainage must be such as to prevent any surface overflow from adjoining property, and also be ample to keep the stock in good condition at all times. It should be ample to care for the waste, as this is sometime a serious problem. If the natural body of water available is not sufficient, settling tanks or filters may be necessary. Fermenting material, such as tomato trimmings or corn refuse, should not be tolerated within or near the factory. The supply of water should be sufficient for all purposes and of good quality; that used in washing, blanching, and brining should be free from excessive hardness or iron, otherwise the finished products may be damaged. If the water for this purpose is not naturally of the right quality, artificial treatment will be necessary. The water used for washing about the factory should have a good pressure for cleaning. A factory with a poor location, or an insufficient or poor water supply, has a handicap which is difficult to overcome. The facilities for bringing in or sending out of stock should be ample, so that materials used need not be delayed, especially when it may mean deterioration.

The buildings should be designed with reference to the special products to be packed, but there are some features which should be common to all. The ceilings of all rooms should be high, with ample provision for light and ventilation. The light should come from numerous side windows, or, if the rooms are large, from turrets, or a saw-tooth-roof construction. Either of these two arrangements can be made to give a flood of light and at the same time provide good ventilation. An advantage in the saw-tooth construction arises from the cooling and drying effect. When the straight section, or windows, are turned toward the north, the sun beating upon the southern incline will heat the layer of air underneath, causing it to rise. This creates a circulation within the room which tends to dry floors and tables and to lower the temperature. Tests made in factories so constructed have shown several degrees lower temperature on hot days than was recorded in factories having the usual form of roof.

One of the marked contrasts between the newer and older construction is the provision for plenty of light. Light has a beneficial effect upon employees, contributes to cleanliness, and is an active, constant disinfectant. High ceilings and proper roof construction usually render artificial ventilation unnecessary, but if mechanical measures are employed a blower system, with provision for cleaning the air, is to be preferred to suction. An abundance of light and air is a combination which will contribute to the maximum of labor efficiency.

A tight, hard floor is a necessity, and in all rooms where manufacturing processes are conducted it should be pitched about 1½ inches for each 10 feet. The pitching should have special reference to the position of machines and tables where there will be more or less water or waste, so that this may be confined and the floors be flushed clean and kept reasonably dry with the minimum of labor. There should be frequent trap connections with the sewer. The kind of material best adapted for a floor will depend in a measure upon whether it is to be used for dry work and storage or whether water is employed more or less freely. Factories having a short packing season, as in the case of tomato canning, find concrete to be the best. Wood shrinks, swells, and cracks with changes of moisture; the cracks are hard to clean, leakage is almost certain to occur, and these conditions become aggravated in factories which are idle a part of the time. Wood with a smooth covering, such as sheet roofing, makes a good floor, but will not last long. Concrete is more or less porous, wears rough, and is not an ideal floor, but is the best for certain conditions. Asphalt wears away and crumbles too easily. Upper floors should not be chosen for food preparation if plenty of ground space is available, for the reason that it is difficult to keep them tight. Furthermore, the work can be supervised to better advantage on one floor than on many, unless the departments are so large as to demand a superintendent in each. Conveyers can be obtained to handle products from one machine to another, and these are more easily kept clean than are floors. Conveyers and overhead tracks should be used in handling the product as far as is possible in preference to trucks, as the latter are destructive of floors and are not so clean.

The use of slat gratings to cover the floor about the kettles or other places where there is a splashing or overflow of water is especially to be commended. These may be made in sections about 2 by 4 feet, and can be taken up for cleaning. There is no excuse for floors being so wet or sloppy that the workers must wear rubbers, which is sometimes the case. All side walls, partitions, ceilings, and supports should be smooth, to admit of easy cleaning. Preferably they should be light colored and, as far as possible, of such material as can be washed with a hose, as this is the easiest method of cleaning or of applying whitewash. Some factories need to be divided by partitions to prevent unnecessary heating by steam from the cookers. In other cases the room where the material ready for the can is kept should be separated from the rooms in which the preparation is going on, in order to protect it from dust. That part of the factory in which prepared material is in any way exposed should be screened to keep out flies and dust. The latter precaution is often of greater importance than the former, for during the working period the moving of machinery and escaping steam will drive away insects.

The tables used in the preparation of foods should be plain and of a material that is easily cleaned. There should be no sharp angles or grooves where waste can accumulate, nor any places beneath where material can be stored. Hard wood, such as maple or ash, is probably the best material for the majority of factories. These woods will absorb little water or juices, they show soil quickly, and clean easily with soap, water, and scrubbing brush. Opal glass or porcelain makes excellent table tops but is expensive. Enamel-coated metal has come into use, and under certain conditions gives excellent results. The important point is that the tables may be cleaned easily, and that it be done often. The machinery used should be of the most sanitary type, and set in such a manner as to be accessible from all sides for cleaning. Conveyers for fruits, tomatoes, and all other products should have automatic washers and brushes in their course to keep them clean. The amount and kind of equipment varies greatly, depending upon the product. Peas, corn, and beans require the most, fruits the least. The details of the special requirements will be considered under each product. Water and steam pipes, with hose attachment, should be conveniently placed about the factory for cleaning tables, machines, floors, walls, and ceilings. This is a necessary part of a modern equipment.

Provision should also be made for the cleanliness and comfort of the employees. Water should be placed at convenient places that the workers may wash their hands often, and sanitary drinking fountains installed to take the place of the common cup. A factory is not complete without proper toilet and clothes rooms. The toilet should have facilities for washing the hands with soap and water and hand brushes should be provided. There should be lockers for storing the outer clothes, as wearing apparel should not be hung about the factory. Providing special suits and a manicurist are refinements which are found at some factories and are not so much of an extravagance as less progressive firms would argue. For factories running continuously and employing the same help, there is no question but that uniforms are advantageous. For such operations as picking, peeling, and pitting fruits, etc., which can be done as well sitting as standing, stools should be provided. Standing all day at tables is more than tiring, it is exhausting and decreases efficiency. This is clearly evident to every factory inspector, especially after the season has advanced. The stool is to be preferred to the common bench, so that the individual may stand or sit as may be most comfortable. If standing in one place over cement floors is necessary, wooden springboards should be provided for the restful effect upon the feet. The various States provide the general conditions under which labor can be performed, age limit, number of working hours in the day or week, physical condition, etc. No person affected with communicable disease should be employed in a food factory.

METHODS AND PROCESSES.

The steps in canning will vary with the product, but, in general, there are certain processes which are common to all and may be described in this outline, as receiving the product, grading, washing, preparing for the can, filling, exhausting, capping, processing, and cooling.

RAW MATERIALS.

The first requisite in all canning is that the product be delivered in first-class condition, fresh from the fields or orchard, and in a manner to prevent injury. Fruits, such as berries, must be handled in boxes as for the market, tomatoes in shallow crates, corn, peas, and beans in such quantities that they will not heat, and marine products cold or chilled and in compartments to avoid bruising. The condition of the material on delivery is of the greatest importance, and for that reason the factory should be located near the point of production, or, if shipment be made, the distance should be short and direct. A cannery which depends upon long-distance shipments or purchasing the supplies on a city market will generally be found to put out an inferior article. In any delivery the seller should be held responsible for the condition of the material; the grower has no more right to deliver decayed tomatoes than the canner has to use and ship them. The first case is usually a violation of a State law and should be dealt with accordingly; the second may be reached by Federal statute if the shipment becomes interstate.

GRADING.

The second step, that of grading or sorting for quality, is one of great importance. A general inspection or classification of all products is made by the foreman at the time of receipt, but this is insufficient. The real grade of any product depends upon the quality of the original stock rather than upon the sirup or brine added or any subsequent operation, and the best time to make a separation is before the work of preparation is begun. A large part of the sorting can be done better by a few especially trained helpers, although some of it may be continued in subsequent operations. The hard and faulty ears of corn can be picked out more easily while it is being conveyed to the silker than by the cutter feeders. These men have enough to do to keep the machines busy and can not take the time to sort properly. A few persons can pick out green, defective, and wrinkled tomatoes which will not peel economically and do a better job before the fruit reaches the scalder than can be done by the peelers. The same principle holds true for peaches and many other products. Those who peel or fill the cans should have the minimum of grading to do. The sorting is usually done upon belts or special table tops to expedite the work. Berries are picked, stemmed, and defects picked out, when graded, to save handling.

WASHING.

The next operation is generally that of washing, the method depending upon the material canned. In general, most products are placed in a tank of water to loosen adherent dust and dirt, are gently rolled over by the agitation of the water, and sprayed as they emerge. The spraying is the important step, therefore, it is desirable that the water have force rather than a large volume. A small spray with force will cut off dirt and adherent mold very successfully. The principle is the same as cleaning a floor with a hose having a nozzle, or with one having an open end; the former will use less water but will clean better. Some hard-coated products, as peas, are washed in revolving wire cylinders, known as "squirrel cages." Soft fruit, such as raspberries, require very gentle washing, and if the fruit appears clean some packers object to washing it at all, claiming that it causes injury and loss of flavor. Whatever method is used, the cleaning should be thorough.

PREPARATION AND BLANCHING.

Many of the fruits need no special preparation other than cleaning and sorting, after which they are placed directly in the cans. Peaches, apples, pears, etc., must be peeled and cut into pieces of the proper size. Nearly all vegetables require more or less treatment; peas are shelled, graded for size and quality, and washed and blanched by automatic machinery; corn is cut, silked, brined, and cooked; beans are snipped and strung, graded for size, and blanched; asparagus is cut into lengths and blanched; sweet potatoes and beets are peeled and graded, and so on. The operation of blanching is in reality parboiling. Vegetables are dropped into boiling water for from one to five minutes, as a rule, to cause softening, and at the same time to remove some of the mucous substances which form upon the surface. The effect produced by a short boiling in the open as compared with boiling in the closed can is surprising. Peas or beans, which are a little aged and hard, will soften quickly in the blanch but retain their condition in the can. In almost any case of very cheap peas some may be picked out which, if thrown upon a table or the floor, will bounce a couple of feet or more. This is evidence that they were not properly blanched, and that softening did not take place in the can. The operation of blanching is of much importance in putting up good vegetables. It is not a matter of whitening, as the name might seem to indicate, though it does have the effect of producing a much clearer liquor than would otherwise be present.

WASHING AND FILLING THE CANS.

The cans should be washed just prior to being used. In the shipping and storing, more or less dirt and dust find lodgment on the inside, and washing is the only method of removing it. The quantity of dirt which can be obtained from a thousand cans is usually a matter of surprise. The work is done very effectively at the present time by machines. The filling may be done by hand or by machine. There are many products, especially fruits, which can not be successfully filled by machine because of crushing or otherwise injuring them. When filled by hand the contents should be regulated by weight rather than by volume, so that the finished product will be uniform. If the filling be done by machine, care should be taken to get the best results possible. It is illogical to use care in peeling a 3-inch tomato and then have it squeezed through a 2-inch opening in front of a crude plunger; or that great care should be exercised in washing and blanching peas, which are to be run through a filler that will cut or crush enough to make a muddy liquor. Machines should be designed to fill with reference to the nature of the product and not to be merely "can stuffers." Vast improvements have been made in filling machines in the past few years, so that most of the work can be done with nicety and precision. All filling machines operate upon the principle of delivering a certain volume rather than a given weight, and for most products this method is very satisfactory. In all cases, whether the can be filled by weight or volume, the amount of material used should be all that can be put in the can in first-class condition. Brining and siruping have also been improved, the old-fashioned unsanitary dip box giving way to a sanitary filler.

EXHAUSTING.

After the can is filled the next step is exhausting. This is best for all articles packed cold, but is unnecessary for corn, peas, or products which enter the can hot or are covered with a hot sirup. Exhausting consists in passing the filled can through a steam box and heating the contents to 160° F. or above, the preference being for 180°. The time required for exhausting will vary from one to three minutes.

CAPPING AND TESTING FOR LEAKS.

Open-top cans are sealed by a special machine known as a double seamer. The lid is pressed into place and steel rollers crimp it on without acid or solder. This action is automatic, a single can at a time, but at the rate of 30 per minute, or 1,800 per hour. Cans with solder tops are sealed by automatic machinery, 12 at a time, 85 per minute, or 5,000 per hour. The top is wiped, the cap placed on, acid applied, the hot soldering irons drop into place, and the vent is afterwards closed, all in one series of operations, without touching by hand. As the cans pass from the capping machine they may be submerged in a bath of boiling water to test for leaks. Any imperfection in the can or defect in sealing will be shown by a series of air bubbles issuing from the opening, and the can is at once taken out by the inspector for repairs.

PROCESSING AND COOLING.

After capping, the cans are processed according to the nature of the contents. The cans are collected in large iron baskets, which usually hold 270 No. 2 or 180 No. 3 cans, and three baskets fill a retort. If the processing is conducted at boiling temperature, the retort is not closed, but steam is turned into the water which covers the cans. If the temperature is to be above the boiling point, the retort is closed and either the steam is turned into the retort until the proper pressure and temperature has been reached, or water is first turned in to cover the cans and the steam is admitted until the temperature has been attained. In processing fruits it is customary to use long vats containing boiling water and equipped with automatic conveyers, which carry the cans or crates through at such a speed as will process them for the necessary length of time. This period varies with the product. Sterilization depends on administering the proper amount of heat, and heating above the required temperature or for longer than is necessary only cooks the material to no purpose.

As soon as the processing is completed, the cans should be cooled with water. Unless this is done, the heat will be held so long that the contents become overcooked—fruits are softened, and tomatoes become liquid, even blacken, peas break and make muddy liquor, while corn acquires a brown color and a scorched taste. The cooling may be done by turning cold water into the retort, by removing the basket of cans to a cooling tank, or by spraying with water in the air. There is less difference in the results obtained by different methods of applying either heat or cold than some claim; the important point is to accomplish these steps quickly.

In canning operations the product, salt, sugar, or other seasoning, and water are the only materials used. No hardener, bleach, or preservative is employed, and in commercial canning there never was as much preservative used as is common in the household operation. Saccharin and sulphites were formerly used in corn and peas, but their use has now been practically discontinued; on the other hand the practice of selling a "canning compound" to housewives still continues, and will only cease when the nature and effects of such chemical preservatives are known, and the lack of necessity for their use is appreciated.

CONTAINERS.

The first container used was the ordinary glass bottle with a comparatively small mouth, and was closed with a cork. The next step was the use of a resinous wax to cover the cork. The bottle was modified to the more convenient or jar form, and a groove run around the top so that a tin cap might be sealed in place with wax. This method of sealing was common in domestic canning until about 1890. The metal screw cap with the rubber ring and various other devices, most of which depend on a rubber or fiber joint to exclude the air, have been introduced since that date. The glass jar is largely used in domestic canning, but not commercially, as it is heavy, breaks easily, can not be handled by automatic machinery, will not stand hard processing without special precautions, and increases freight rates. Glass containers are used for preserves, spiced and pickled fruits, and for the limited canning for which the consumers are willing to pay a fancy price. Very recently improvements have been made in glass jars and the methods of sealing, which may extend their usefulness, especially to such products as can not be preserved to the best advantage in tin.

The earthenware jar was brought out to offset the high cost of the glass jars; some of these were glazed inside, some outside, and some on both sides. They were generally sealed with a tin cap by means of wax, though a few had earthen tops. Various forms were given to these jars, and some may still be found which have been in use for many years in rural districts. The earthenware jars had only one advantage over glass, that is in cost, but they had the disadvantage of having blow or sand holes. The earthenware jar is not used to any large extent in commercial canning, though some are used to pack bulk jams and stock for preserves, etc.

The tin can is preeminently the container used in commercial canning, and it is also used to a very large extent in home canning. Those used for the latter purpose retain the deep ring about the opening for the insertion of caps and sealing with wax; these are commercialy known as wax-top cans. In commercial canning solder is used exclusively for sealing stud hole or cap cans. The tin can has undergone a number of changes. The first cans had flush sides and ends, or plumb joints; these gave way to the stamped-overlapped ends, and all inside soldering has been superseded by lock seams and outside soldering. Most solder caps are hemmed, so that only the amount necessary to seal is used. The solder can has been superseded in many cases by the open top, or so-called sanitary can, and in this case the sealing is done by double seaming on the top, no solder being used on the can except in making the side seam. The former objections to acid and solder, on the ground that they contaminated the foodstuffs, have thus been largely overcome.

The most recent improvement in the tin can is the inside coating or lacquering. This type of can is known to the trade as the "enamel lined" can. Various coatings have been tried at different times without entire success, and while the present lining is not perfect, it does effect a marked improvement in many lines of packing. There are fruits and vegetables which attack the tin coating with more or less vigor, resulting in a loss of color, flavor, and quality, and at the same time form salts of tin which are objectionable. The inside lacquered cans are especially effective in holding such articles as raspberries, cherries, plums, beets, pumpkin, hominy, etc. They do not add to such products as corn, peas, beans, tomatoes, or those which have little action upon the tin. Inside coating is accomplished in two ways—by baking the lacquer on the sheet and by spraying it on the inside of the finished can; further improvement in the container may be expected along these lines. The tin can is made in a great variety of sizes and shapes, but there are certain forms known as standard.

Sizes of standard cans.

Number of can.	Diameter in inches.	Height in inches.	Capacity in ounces.
1	2 11/16	4	11.6
1 tall	2 11/16	4½	12.3
2	3⅜	4 9/16	21.3
2½	4	4¾	31.2
3	4 3/16	4⅞	35
3 tall	4 3/16	5½	39
8	6 3/16	6⅞	104
10	6 3/16	6 9/16	107

The size of package used for certain products is fixed by trade custom and not by the needs of the consumer. For example, corn, peas, beans, and such products are almost exclusively packed in No. 2 cans, tomatoes in No. 3, and California fruits in No. 2½ cans. The No. 2 can of high-grade peas or corn contains about 22 ounces, or too much for one service for a family of two, three, or four persons, and with peas in particular the unused portion is not so good when served a second time. A can holding 16 ounces would more nearly meet the requirements. The same is true for a No. 3 can of tomatoes. The excess is waste in many cases and represents not only good material but the labor expended upon it, a larger can than is necessary, and boxing and freight. These are all items which contribute to cost and a consequent lessening of the use of canned foods. The No. 2½ can was developed as a short weight from the No. 3 and does not adequately represent the interval in size between the No. 2 and the No. 3. The No. 2½ sanitary can holds only slightly less than the No. 3 in the older style, as the latter can not be filled so nearly full and sealed. Recently a new style of can has been introduced for California fruits, especially for peaches, known as the luncheon size, which is one-half the height of the No. 2½. These are desirable because they will take in the large pieces of fruits and apparently are meeting a demand. The same style in the square can is being used for asparagus tips.

At the present time some packers are trying to meet certain demands by varying the fill rather than the size of the can. For example, a well-filled can of tomatoes might retail at 15 cents, the packer may reduce the quantity, add water, and make the cans sell two for a quarter, or carry it to an extreme and sell for 10 cents. A customer finding that the 10-cent can will furnish the amount of tomato wanted and without waste will repeat the order. The same methods are used more or less in packing fruits, using a quantity which will make the can sell for a certain price. This is a crude, unsatisfactory, and manifestly expensive method, and also open to fraud by those who are unscrupulous. It would be far better for the packer to determine what size is wanted and use such sizes, filling them properly.

THE LABEL.

The label should tell the truth in terms which are direct and easily understood. It should give the name of the article, the grade, by whom packed and where packed, or the name of the distributor. Neither the names nor the illustrations used should be misleading. A picture of green peas in pods in clear relief and subdued type stating that the contents are soaked is hardly appropriate. If given a geographical name it must be the true one. Corn grown in Iowa is not Maine corn though obtained from Maine seed. The use of such terms as " Maine style " for cream corn is in reality only an attempt to circumvent the intent of a true label.

There are no fixed standards for canned goods, though the canner and the trade do recognize and describe certain qualities in jobbing, and prices are made accordingly. The consumer has not been educated to know these differences. The labels usually carry descriptive terms implying superlative quality, as extra select, extra choice, extra fancy, select, choice, fancy, extra standard, and, less commonly, standard. There are too many designations for the same product, and, furthermore, Mr. A's fancy may not be the same as Mr. B's. The grade may not be the same in two consecutive seasons, due to drought, excess of rain, intense heat, or other cause; neither may it mean the same in different sections of the country in a normal year. In other words, at the present time the grade does not have a fixed character.

Again, when the sirup is one of the factors in grading a product, that fact should be given, though it is not required. A consumer can not go to the grocery and buy peaches in a 40°, 30°, or 20° sirup, though the packers use care in preparing such sirups to use for their different grades. Such designations as heavy, medium, and light sirup are also inadequate. A heavy sirup may mean anything between 35° and 60°, a medium between 20° and 45°, and a light between 10° and 30°, depending on who uses it. These variations are too wide to be carried under such elastic terms. There is no doubt that some fruit packed in light or 20° sirup is just as good as that put up in medium or 30° sirup, but there can be no harm done by giving the exact facts. On general principles, if it is worth while for the packer to select his stock carefuly and put up different grades, the consumer should know how to select them.

A can of any food should be as full as it can reasonably be packed and processed without injuring either the quality or appearance of the product. There is such a thing as overfilling as well as under filling, and one is as much a fault as the other. All foods packed in a liquid or semiliquid condition, or as solids surrounded by liquid, should fill to within one-half inch of the top, and when free liquid is present it should cover the solids. Corn or peas an inch below the top would be a slack fill, even though covered with liquid. The fruits present a more perplexing problem, depending upon the size of the pieces and the degree to which they shrink in the sirup. The very choice large peaches, having only 5 or 6 pieces to the can, will weigh only 18 or 19 ounces and be as full as they can be sealed. A slightly smaller size, of 7 to 9 pieces to the can, will weigh 20 ounces, and for more than 10 pieces the weight will be from 21 to 22 ounces. After they have been cooked in the sirup the pieces will soften, the weight will change, and the fill will not be the same, though in all the amount was as much as could be sealed. If the cans be judged upon weight of the solids alone, the highest grade would be short weight; the quality must also be considered. The presence of only 18 or 19 ounces of low-grade peaches would be manifestly slack filled. Soft berries, like strawberries and raspberries, if filled as full as the can will hold and sirup or water added, will appear only one-third to one-half full of solids upon opening and considerable variation will occur, depending upon their condition. Some foods can be packed so as to give a fairly uniform net weight upon opening, but with others the volume of solids and its own liquid is a fairer measure. The buyer is entitled to a full can and most packers try to furnish it. The net weights given for several products at the close of the descriptions of processing are intended to represent the minimum; the amount actually obtained should exceed these figures. A lower net weight may be regarded as "slack filled."

USE OF THE TERM "CANNED."

The term "canned" as applied to food products put up in hermetically sealed packages is capable of more than one meaning. Originally it meant any food put up in any container which might be hermetically sealed and the preservation accomplished through sterilization by heat. In commercial use the term "canned" applies only to foods put up in tin containers and sterilized by heat. Under that construction any foods put up in glass or other containers than tin are not rated as canned foods, nor are foods put up in tin in which preservation is accomplished by some means other than heat. Fish cured in brine, pickled, or spiced, but packed in tin, is not canned within this meaning of the term. Fruits preserved with sugar, placed in glass or tin jars, and sealed in vacuum are not canned in the commercial sense. The same is true of smoked meats, such as dried beef, and fish, as smoked herring. In domestic canning glass jars are generally used, and the product is referred to in the home as canned. It is unfortunate that the term should have so many meanings. In the trade it is now common to refer to fruit in glass, sliced bacon and chipped beef in glass or tins, sliced or smoked fish in glass or sardines in tins, and candied fruits in glass.

SPOILAGE.

Spoilage may result from insufficient processing, defective containers, or the use of unfit material. These losses are generally classed under the heads of swells, flat sours, and leaks. Formerly losses were heavy at many factories, but these are becoming less each year, due to a better knowledge of what is necessary in material, handling, and improved appliances. More attention is paid to testing for bacteria, and greater care is taken in obtaining accurate thermometers and gauges, automatic temperature-regulating devices, and time recorders, so that little is left to the judgment of the processor or helper.

Spoilage due to insufficient processing is generally divided into two classes—swells and flat sours. In the former there is generation of gas, causing the ends of the can to become distended; in the latter the content of the can is sour, but there is nothing in the appearance of the can to enable the customer to determine the condition until the can is opened. Swells are generally due to underprocessing good material, while flat sours most often result from giving the regular process to material which has been allowed to stand for some time, such as peas remaining in a load overnight or corn left in a car or in a pile until it begins to heat. The raw material may show no evidence of fermentation on superficial examination, but this condition frequently exists under the conditions just cited. Swells are therefore more likely to be associated with rush operations and flat sours with an overstock or delay in getting at the raw material. It is not intended to give the impression that swells and sours may not occur under other conditions, such as changes in the consistency of the corn, nor that swells may not occur in material which has stood, and sours result from underprocessing, but only to state a general rule.

Swelling or souring may take place shortly after processing or the spoilage may be delayed for weeks or even months. Swelling is more likely to occur and be detected early, while souring is apt to be delayed, though it may occur early. The heat used in processing may have been insufficient to kill the vegetative forms or spores, but may have injured them to such an extent that time was necessary for recovery and subsequent development. A microscopic examination of the material a few days after processing, or of the incubating cans during a short period, might not show anything wrong. It is only by incubating samples for a number of days that early recognition can be made of some cases of spoilage or possible spoilage. The canner often sends his goods from the factory with full confidence in their condition, and it is not until after they have been in the broker's warehouse or upon the grocer's shelves many weeks or even months that he becomes aware that anything is wrong. The spoilage may amount to only one can to the case, or the percentage may be high; but in either event the goods are rejected with loss.

Spoilage from the use of improper material—i. e., material which has been allowed to stand until fermentation has begun—is generally more or less sour to the smell and taste, but is sterile, the heat of processing having killed the bacteria.

Can leaks may occur along the side, "seam leaks" ; at either end, "end leaks"; at the cap, "cap leaks"; at the tip, "tip leaks"; or may be due to defective tin plate. Can making has reached such a point of perfection that manufacturers guarantee all above two to the thousand. These imperfect cans are usually due to the solder not making a perfect union or to defects in crimping or double seaming. With the use of the automatic capping and tipping machines there are fewer leaks than formerly occurred when the work was done by hand; leaks in sanitary cans are generally due to poor adjustment of the rollers. Leakers are recognized, as a rule, by inspection in the hot bath, few getting into the wareroom. Leaks may be very small, even microscopic in size, and, therefore, difficult to detect, or pieces of the can content may be driven into the opening and seal it for the time. Leaks invariably cause swells. A check on spoilage can be kept by placing a few cans from each day's run in a room kept at a high temperature (98°), as these will incubate much more rapidly than if kept in a storeroom.

There are two conditions, known to the trade as "springers" and "flippers." A springer is a can the end of which will bulge slightly after a time, but on opening there is found neither gas nor spoilage, though the cans have the appearance of being swells. This condition has been found to be due to overfilling or to packing cold. Such goods when placed in a warm grocery will bulge, due to the temperature. A flipper is a springer of such mild character that the head may be drawn in by striking the can on a hard object. It is always possible to tell a swell from a springer by the use of a microscope, as in the former there will be large numbers of organisms while in the latter there will be very few.

While a spoiled can of food should never be eaten, the danger of poisoning from fruits and most vegetables is very remote. Ptomains or other poisons may form in meat, milk, and fish, but rarely, if ever, in vegetables.

EFFECT OF HEAT AND COLD.

Canned foods may be injured by an excess of either heat or cold. Some products are injured more than others. The effect of prolonged heating is to cook the contents to a pulp. This is seen at times, in the case of peas and tomatoes in particular, when the cans have been stacked tightly before being fully cooled. The liquor will become cloudy from short heating, thick and heavy from prolonged heating, and the peas softened and broken if it is continued for a number of days. The writer has seen peas stacked that were warm for three weeks after packing. Tomatoes become soft and pulpy, and often turn a walnut brown if stacked hot and the heat is retained. All fruits become murky and lose their distinctive flavor and odor. Canned foods will stand the high temperature of summer very well, but as far as possible they should not be placed in the hot sun nor kept in a very hot storeroom. The effect of moderate heat is not nearly so marked as might be expected.

Cold seems to have no ill effects upon canned goods unless it goes below the freezing point. Most canned foods will stand a little freezing without appreciable change. Repeated freezing and thawing cause the goods to become flabby and give a flat taste. In all cases the interior of the cans shows a distinct attack upon the tin. With fruits, the coating of the cans is made to appear as though it were galvanized. Canned foods will resist a fair degree of heat or cold without serious injury, but continued heat or a very high temperature, or repeated freezing and thawing will cause deterioration in quality.

Foods properly prepared and kept under reasonably good conditions deteriorate very slowly, so that cans carried from one year to another may be as good as, or better than, the latest pack, depending upon the comparative quality of the fresh product used. On general principles, however, it is desirable that a product should not be carried over several seasons. The amount of tin dissolved also increases with time, which is an additional reason for not holding canned goods any longer than is absolutely necessary.

HOME CANNING.

It is not possible to accurately estimate the amount of home canning that is being done, but it must aggregate many millions of cans. In the rural districts in particular it is considered to be a part of the season's work to put up canned foods for the winter, and from 50 to 100 cans is no unusual stock for a family. The products canned are usually fruits and berries, as these are the most easily handled under home conditions.

The household department of numerous weekly papers gives much space to instructions in home canning, and many cook books give recipes and details of the operation. There are also manufacturers of small home-canning equipments who give glowing accounts of the profits to be made from doing such work. Whether it is profitable to can for home consumption depends upon the cost of the raw material, fuel, and labor. It may be said that it is not generally profitable to buy fruits or vegetables in a city market and put them up in cans. Lots of a bushel, half bushel, or crate generally lack the necessary freshness, are in too small a quantity to permit of grading, and there is too much waste. The labor involved is disproportionately large for the amount handled, and the expense for cans and sugar must not be omitted in determining the cost of the finished product. Home canning may be profitable when the raw stock costs little or nothing, when no account is taken of the labor, and the satisfaction of having one's own handiwork is worth more than the money value of the article.

Home commercial canning is being encouraged to a certain extent and whether it will prove profitable or not will depend upon local conditions. The outfit needed for canning most fruits and tomatoes is very small and where a crop can not be marketed except at a very low price or the labor can not be otherwise advantageously employed, a fair profit may be obtained. The canning of special articles or putting them up in a certain way for an established trade is often successful, but on standard articles like tomatoes, corn, peas, string beans, etc., the chances of home canning in competition with a modern factory are about the same as those of a hand meal grinder as compared with a modern grist mill. The product of the average home cannery will grade in quality on about the same par as country butter. Both depend upon the producer, but as a class neither ranks very high. The small home cannery is useful in saving good food which would otherwise go to waste, and its development should be encouraged, but the idea of large profits should be held in abeyance. For the novice and many others it would be better to learn how to buy the best prepared foods rather than to attempt to pack them.

COST OF CANNED FOODS COMPARED WITH FRESH.

In making a comparison of the cost of canned and fresh products of the same kind, a number of factors must be taken into consideration. First, the cost of the raw material and the waste when purchased in the small quantity used in a single meal; second, the cost of labor and preparation used in making it ready for the table. It is obvious that a comparison can not be made for time, as the canned article may be had throughout the year and the fresh for only a limited season, and purchase of a product out of season is usually at a high cost. In making a purchase of either the fresh or canned article, the smaller the quantity, the higher the price; the single can costs more than if bought by the dozen or case, as does the half peck of apples compared with the bushel or barrel.

Take, for example, a No. 3 and a No. 10 can of whole apples; the former usually retails for 10 cents and the latter for 25 to 30 cents. Those who can use the latter have a decided advantage, as it will contain between four and five times as much as the former. Only in apple districts, and for short seasons, can the same quantity of the fresh fruit be purchased at the same price. Wherever the fresh fruit sells at the rate of $2 per bushel when purchased by the peck, and this is below average prevailing prices in cities, the canned article is the cheaper. In the raw stock there is loss in peel and core, from bruises, short weight, and often rot, all of which is eliminated in the can. The canned variety usually cooks better, and for the pie or dumpling is generally the cheaper.

Neither corn nor peas can be purchased in large cities, nor in many smaller ones, as cheaply as in the can, and then they are not so fresh. In up-to-date canneries the article is put up the day it is picked, while 3 or 4 days may elapse from the time the raw product is harvested in the garden (in transportation, in the hands of the commission house, and in the grocery) before it reaches the consumer's table. It requires nearly 2 quarts of good peas in the pod to make one can, and often more than 3 quarts of the heavy-podded variety found on the market. At no time can the smaller peas nor fine-kerneled corn be purchased as cheaply as in the can. It requires 2 bushels of good peas to yield one No. 2 can of petit pois, or 1 bushel to yield one can of extra sifted, and from 4 to 8 ears of small corn to make one can. The pea and corn packer, however, handles tons of these crops especially grown for him, and uses the highest class of automatic labor-saving machinery in all operations, so that the real labor on a single can is very small. The consumer can not purchase peas at from 30 to 50 cents per bushel, nor corn at $9 to $12 per ton, and these represent initial costs in large quantities.

In fruits, as berries, the consumer must figure that a No. 2 can will require not less than 12 ounces of well-selected fruit, and for a No. 2½ can (22 ounces). The latter is equivalent to two boxes of berries by the time they are picked over. Sirup is added to the can, which offsets the sugar necessary for the fresh fruit.

There is a vast difference in canned foods, and, as in many other lines of commerce, the cheapest in price is often the most expensive. The can of water-packed tomatoes, the green hard pears, the handful of berries in a pint of water, or poor-quality beans disguised with tomato dressing and offered at a low price, when measured by their food value are the highest. Goods which are strictly standard should give the best food value for the cost. Peas, corn, beans, and tomatoes which are good field run, but which lack the uniformity and niceties which are necessary for the fancy article, will have all the nutritive properties, and be just as palatable, but cost several cents less per dozen. There is much that is pure fad in the purchase of canned foods; the asparagus must be white and the fewest possible stalks in a can; the green is just as good and a medium number of stalks furnish a more edible product. The little peas are, naturally, the costly ones, for less than 5 per cent are of that kind; the large ones are the better flavored and more nutritious, and one-third the cost. Similar examples might be cited of a number of other products. Canned foods should be purchased by the dozen or case, straight or in mixed lots, rather than by single cans.

EXTENT OF THE CANNING INDUSTRY IN THE UNITED STATES.

The figures presented by the Bureau of the Census give one a general idea of the importance of the canning industry. In 1909, there were 3,767 establishments engaged in canning and preserving, the capital invested was $119,207,000, that paid for raw material was $101,823,000, and the finished product was worth $157,101,000. The number of cases of the principal vegetables was as follows: Tomatoes, 12,883,414; corn, 7,447,765; peas, 5,873,748; beans, 3,774,923; and all others 3,093,493. The number of cases of the principal fruits was as follows: Peaches, 1,479,601; apples, 1,169,730; berries, 792,244; pears, 628,485; apricots, 562,811; and all others, 717,144. The number of pounds of fish was: Salmon, 99,831,528; sardines, 90,694,284; oysters, 28,192,392; and all others, 16,700,509. The total number of pounds of condensed milk was 494,796,544.

The accompanying table, which shows the time and place of canning the principal fruits and vegetables, is not complete, but was made up from the reports of those canners who replied to the series of questions addressed to them.

Seasons for packing various products in the different States.

State.	Apples.	Apricots.	Asparagus.	Baked beans.
Arkansas	July 23 to Aug. 15			
California	Sept. 17 to Nov. 26	June 1 to Aug. 10	Mar. 25 to July 1	
Colorado	Sept. 1 to Oct. 31		May 1 to June 30	
Connecticut	Sept. 30 to Oct. 30			
Delaware	Sept. 20 to Oct. 10			
Georgia	Aug. 1 to Sept. 1			
Illinois	Aug. 10 to Nov. 15		May 20 to June 20	Jan. to Dec.
Indiana	Oct. 1 to Nov. 15			do.
Kansas	Oct. 1 to Oct. 28			
Maryland	Oct. 1 to Nov. 1			Jan. to Dec.
Massachusetts				do.
Michigan	Aug. 1 to Nov. 1			do.
Minnesota	Aug. 15			do.
Missouri	Sept. 18 to Oct. 17			
Nebraska	Sept. 1 to Nov. 1			
New Jersey			May 13 to July 1	Jan. to Dec.
New Mexico	Oct. 15 to Dec. 25	Aug. 1 to Aug. 15		
New York	Sept. 15 to Dec. 31	July 20 to Aug. 20	May 10 to July 15	Jan. to Dec.
Ohio	Oct. 1 to Nov. 20			
Oregon	Aug. 25 to Dec. 1			
Pennsylvania	July 1 to Dec. 1			
Tennessee	Oct. to Nov.		Apr. 15 to May 10	
Utah	July 25 to Dec. 1	July 24 to Oct. 1	Apr. 26 to June 10	
Virginia	Sept. 1 to Oct. 20			
Washington	Aug. 15 to Dec. 10	July 1 to Aug. 1		

State.	String beans.	Beets.	Blackberries.	Cherries.
Arkansas			July 1 to Aug. 15	
California	Aug. 1 to Sept. 15		May 29 to Sept. 10	May 15 to July 28
Colorado				June 15 to Aug. 1
Delaware			July 1 to July 20	
Georgia	July 1 to Aug. 1		June 10 to June 20	
Illinois				June 15 to July 15
Indiana	June 1 to Oct. 1			
Kansas	June 8 to July 27			
Maryland	July 10 to Aug. 20		July 4 to July 20	June 8 to June 30
Michigan	June 10 to Sept. 15	July 1 to Oct. 1	July 15 to Aug. 24	June 25 to Aug. 10
Minnesota		June 20 to Oct. 22		
Nebraska	June 10 to July 15		July 1	July 1
New Jersey		June 15 to July 25	July 5 to July 15	June 9 to June 20
New York	July 1 to Oct. 28	July 15 to Nov. 25	July 23 to Sept. 1	June 20 to Aug. 1
Ohio	July 1 to Sept. 30	June 25 to Nov. 10	July 1 to Aug. 10	June 1 to June 30
Oregon	July 15 to Oct. 15		July 15 to Oct. 15	June 10 to Aug. 20
Pennsylvania	July 10 to Oct. 15	Aug. 1 to Sept. 15		Aug. 17 to Oct. 1
Tennessee	June 15 to July 10		June 15 to July 5	May 25 to June 25
Utah	June 30 to Oct. 1			July 1 to Aug. 15
Vermont	July 20 to Aug. 20			
Virginia	July 20 to Aug. 30		July 1 to Aug. 1	June 1 to June 30
Washington				June 25 to July 20
Wisconsin	July 10 to Aug. 25			

State.	Corn.	Currants.	Gooseberries.	Grapes.
California		June 5 to June 30	May 21 to June 1	Aug. 1 to Dec. 1
Colorado		June 15 to Aug. 30	May 15 to June 30	
Delaware	July 15 to Sept. 15			
Illinois	Aug. 1 to Oct. 1			
Indiana	Aug. 1 to Oct. 15			
Iowa	Aug. 5 to Oct. 1			
Kansas	July 24 to Sept. 15			
Maine	Aug. 20 to Sept. 20			
Maryland	Aug. 1 to Oct. 20			
Massachusetts				
Michigan	Sept. 1 to Oct. 1	July 1 to Aug. 1	June 20 to July 30	
Minnesota	Aug. 1 to Oct. 1		June 1 to July 1	
Missouri	Aug. 10 to Sept. 27			
Nebraska	Aug. 1 to Oct. 1			
New Hampshire	Aug. 25 to Sept. 20			
New Mexico				Sept. 15 to Oct. 1
New York	July 26 to Oct. 17	July 1 to Aug. 5	June 20 to Aug. 1	
Ohio	Aug. 1 to Nov. 1		June 10 to June 20	
Oregon		June 1 to July 15	June 1 to July 10	Sept. 15 to Oct. 30
Pennsylvania	Aug. 15 to Oct. 15			
Utah			June 15 to July 10	
Vermont	Aug. 25 to Sept. 25			
Virginia	July 20 to Oct. 20		June 1 to June 30	
Wisconsin	Aug. 10 to Oct. 10			

Seasons for packing various products in the different States—Continued.

State.	Hominy.	Lima beans.	Okra.	Peaches.
Alabama				July 15 to Aug. 15
Arkansas				Aug. 1 to Oct. 1
California				June 25 to Oct. 25
Colorado	Jan. to Dec.			
Delaware			Aug. 15 to Sept. 15	July 25 to Aug. 15
Florida			June 1 to July 1	
Georgia				June 20 to July 25
Illinois	Jan. to Dec.			Sept. 10 to Oct. 10
Indiana	Jan. to Dec.			
Louisiana			July 20 to Aug. 30	
Maryland		Aug. 1 to Sept. 1	Aug. 10 to Aug. 30	
Michigan		Aug. 15 to Sept. 20		Sept. 11 to Nov. 1
Missouri				Aug. 11 to Sept. 5
Nebraska				Sept. 10 to Oct. 10
New Jersey		Aug. 1 to Sept. 30	June 1 to Sept. 20	
New Mexico				Sept. 1 to Oct. 1
New York	Jan. to Dec.	July 29 to Oct. 15		Aug. 25 to Oct. 20
Ohio	Jan. to Dec.	Aug. 10 to Oct. 30		Aug. 10 to Aug. 31
Oregon				Aug. 10 to Oct. 10
Pennsylvania	Jan. to Dec.	Aug. 15 to Sept. 15		
Tennessee				July 20 to Aug. 20
Texas				June 15 to Sept. 1
Utah				Sept. 6 to Oct. 6
Virginia				Aug. 1 to Oct. 15
Washington				July 15 to Sept. 30

State.	Peas.	Pears.	Pineapples.	Plums.
California	May 20 to June 20	July 1 to Oct. 27		July 1 to Sept. 10
Colorado		June 15 to Aug. 15		
Connecticut			Sept. 30 to Oct. 20	
Delaware	June 1 to June 30	Sept. 20 to Oct. 20		
Florida			May 15 to Sept. 1	
Georgia	June 1 to June 15			
Illinois	June 14 to July 14			
Indiana	May 26 to July 15			
Kansas	June 5 to June 30			
Maryland	June 5 to July 1	Sept. 1 to Nov. 1		
Massachusetts		Oct. 1 to Nov. 1	June 2 to June 10	
Michigan		Aug. 20 to Nov. 5		
Minnesota	June 15 to Aug. 1			
New Jersey	June 6 to June 25	Oct. 10 to Nov. 15		
New Mexico		Sept. 15 to Oct. 15		
New York	June 15 to Aug. 31	Aug. 25 to Nov. 9	May 14 to June 25	Aug. 5 to Sept. 20
Ohio	June 1 to July 10			
Oregon	June 1 to July 20	Aug. 25 to Oct. 10		
Tennessee		July 25 to Oct. 25		
Texas	July 1 to Sept. 1	July 15 to Aug. 30		
Utah	June 10 to July 25	Aug. 26 to Sept. 18		
Virginia	May 20 to June 19	Sept. 1 to Oct. 15		
Washington		Aug. 1 to Oct. 15		
Wisconsin	June 15 to Aug. 28			

State.	Pumpkin.	Quince.	Raspberries.	Rhubarb.
Arkansas	Oct. 15 to Nov. 15			
California	Sept. 15	Sept. 6 to Nov. 2	June 28 to Oct. 6	
Colorado	Oct. 1 to Dec. 31			May 15 to June 30
Delaware	Oct. 10 to Oct. 20			
Illinois	Aug. 10 to Nov. 20			
Indiana	Oct. 1 to Nov. 20			
Kansas	Oct. 1 to Nov. 24			
Maryland	Sept. 10 to Oct. 10			
Massachusetts		Oct. 1	July 3 to July 18	
Michigan	Oct. 1 to Dec. 25		July 1 to July 15	July 1 to Aug. 1
Minnesota	Sept. 25 to Nov. 7		Sept. 1 to Oct. 1	June 1 to July 1
Missouri	Sept. 15 to Nov. 15			
Nebraska	Oct. 1 to Nov. 1			June 1 to July 1
New Jersey	Sept. 1 to Nov. 1			
New Mexico	Nov. 1 to Nov. 15			
New York	Sept. 10 to Nov. 13	Oct. 1 to Dec. 1	June 25 to Aug. 15	May 15 to July 1
Ohio	Sept. 25 to Nov. 24		June 7 to July 20	
Oregon	Aug. 15 to Dec. 1		June 15 to July 15	June 1 to July 30
Pennsylvania	Sept. 20 to Nov. 30			
Tennessee	Oct. 15 to Nov. 15		June 15 to July 8	
Utah	Oct. 15 to Nov. 15		July 15 to July 30	May 15 to June 30
Virginia		Sept. 1 to Sept. 30	June 1 to June 30	
Wisconsin	Oct. 10 to Nov. 12			

Seasons for packing various products in the different States—Continued.

State.	Sauerkraut.	Spinach.	Squash.	Strawberries.
California			Nov. 1 to Nov. 13	July 16 to Sept. 28
Colorado	Oct. 15 to Mar. 31			May 30 to June 30
Connecticut			Sept. 30 to Nov. 20	
Delaware			Oct. 10 to Oct. 20	June 6 to June 30
Georgia	Sept. 1 to Dec. 30			
Illinois	Sept. to Nov.			
Indiana	Sept. 1 to Apr. 1			
Kansas	Sept. 1 to Dec. 1		Nov. 2 to Nov. 24	
Maryland				June 20 to July 4
Massachusetts				June 1 to July 8
Michigan				June 15 to July 15
Minnesota	Dec. 1 to Jan. 1	June 15 to July 1		
Nebraska		June 1	Oct. to Nov.	
New Jersey		Sept. 15 to June 25	Sept. 20 to Oct. 30	June 1 to June 21
New Mexico	Dec. 26 to Feb. 1			
New York		June 10 to July 1	Sept. 15 to Dec. 1	May 30 to July 15
Ohio	Sept. 11 to Nov. 15	May 25 to Nov. 30	Oct. 1 to Nov. 10	May 25 to June 30
Oregon			Sept. 15 to Dec. 1	June 6 to July 20
Tennessee				June 1
Texas				July 1 to Sept. 1
Utah	Aug. 1 to Oct. 20		Oct. 1	

State.	Succotash.	Sweet potatoes.	Tomatoes.
Alabama		Nov. 1 to Dec. 1	Aug. 1 to Oct. 10
Arkansas			Aug. 1 to Oct. 1
California			Aug. 8 to Dec. 1
Colorado			Aug. 20 to Oct. 1
Connecticut			Aug. 15 to Nov. 1
Delaware		Oct. 6 to Oct. 18	Aug. 1 to Oct. 20
Georgia		Aug. 1 to Sept. 1	Aug. 10 to Oct. 1
Illinois			Aug. 10 to Oct. 20
Indiana			Aug. 1 to Nov. 1
Iowa			Aug. 10 to Oct. 15
Kansas		Oct. 8 to Oct. 26	July 27 to Oct. 5
Kentucky			Aug. 1 to Sept.
Maryland	Aug.	Oct. 10 to Nov. 1	Aug. 20 to Oct. 20
Massachusetts			Sept. 1 to Oct. 1
Michigan			Aug. 15 to Nov. 1
Minnesota			Sept. 1 to Oct. 10
Mississippi			July 20 to Oct. 30
Missouri			Aug. 20 to Oct. 1
Nebraska	Sept. 1		Sept. 1 to Oct. 5
New Jersey		Oct. 1 to Nov. 1	Aug. 15 to Oct. 25
New Mexico			Aug. 1 to Nov. 1
New York	Aug. 15 to Oct. 15		Aug. 1 to Nov. 2
Ohio	Aug. 1 to Sept. 15		Aug. 10 to Nov. 15
Oregon			Sept. 1 to Nov. 1
Pennsylvania	Feb. 15 to Sept. 15		Aug. 1 to Nov. 1
Tennessee		Oct. to Nov.	July 15 to Oct. 15
Texas		July 1 to Sept. 1	June 15 to Sept. 1
Utah			Aug. 7 to Oct. 30
Virginia			Aug. 15 to Oct. 15
West Virginia			Aug. 1 to Nov. 7
Wisconsin			Aug. 15 to Oct. 1

DETAILED CONSIDERATION OF THE VARIOUS PRODUCTS.

FRUITS.

General Discussion.

Fruits are the easiest of all articles to can, boiling for a short period being sufficient to sterilize in nearly all cases. Formerly it was the practice to pack all fruit in No. 3 cans, but within the past few years a change has taken place; eastern fruits, especially the high grade, are put up in No. 2 cans. The apple is the one exception to the rule. California fruits are packed in No. 2 and No. 2½ cans, the No. 3 can being almost wholly supplanted by the smaller size. In displacing the No. 3 can, the open-top can has been substituted for the solder top, with the result that the cans may be filled with less injury to the fruit and may be sealed full. The quantity obtained in the No. 2½ open-top can is in most cases equal to or more than was obtained in the solder-top No. 3, and it is in better condition.

In the canning of fruits the general practice is to fill the can level full, or nearly so, without crushing, and then add the necessary sirup. The sirup will abstract a certain amount of juice, so that the can will not be full upon opening, and this condition will vary with the different fruits. The softer the fruit, such as strawberries, the less will be the fill, while hard fruits, such as pears, will be scarcely affected. The question of fill will depend in part upon the variety of the fruit used, the state of maturity, the density of the sirup, and the time of processing. The proper selection and handling of the fruit so as to get a can with all the desirable qualities distinguishes the real canner.

The weight of fruit used in a can will vary somewhat when the fill is made by volume, as the interspaces in the case of large and small fruit or soft and hard are not the same. In order to secure greater uniformity, it has been proposed by one of the packers' associations to fill all fruits by weight, 21 ounces for a No. 2½ and 22 ounces for a No. 3 can, before cooking. This is a fair average fill for small or sliced fruits, but peaches in large pieces or whole pears, plums, etc., will weigh less.

In the packing of high-class fruits sirup is used, and this may vary from a very light to a heavy sirup, or between 10 and 60 degrees. Most fruits require the addition of sugar before they are used, and it should be added during cooking, and in canning it has a great deal to do with the development of the proper flavor. The water pack is used only upon the poor grades, or pie stock. The amount of sugar used will depend upon the acidity of the fruit and the flavor desired. It is unsafe to follow a rule-of-thumb method to get the highest class goods; and as the real flavor will not develop until the foods have been put up for some weeks, it requires an expert to determine the proper sirup.

APPLES (PYRUS MALUS).

Apples used for canning should be of such varieties as cook well. They should be slightly acid, smooth and sound, and without bruised spots. Poor apples can not be used in canning and make a first-class product. The peeling is done by hand or power peelers and the core removed by the same operation or with a coring machine. Apples which are intended for dumplings are left whole and graded into size to give a certain number to the can, but those intended for pies or other cooking purposes are sliced in quarters or smaller pieces. The peeled apple is placed in cans as quickly as possible and hot water added to make the fill. If the apples can not be packed in the can at once, they are held in tubs of cold water to prevent their oxidizing or turning brown. The process on apples is about 8 minutes at 212° F. for No. 3 cans and about 10 minutes for No. 10 cans.

APRICOTS (PRUNUS ARMENIACA).

Apricots are produced almost exclusively for canning in California. They are grown and handled the same as peaches, though not quite so carefully, and are graded for size by running over screens having openings of forty, forty-eight, fifty-six, sixty-four, and sixty-eight thirty-seconds of an inch, respectively. The ripe apricot is not peeled, as a rule, but the skin is well wiped either by hand or machine, after which the fruit is pitted. The canning operation is the same as for the peach, though the sirup used is generally lighter. (See Peaches.)

BLACKBERRIES (RUBUS VILLOSUS).

Blackberries should be given the same kind of treatment as raspberries, though they are more solid and will stand being handled in larger volume. They do not require so heavy a sirup. (See Raspberries.)

CHERRIES (PRUNUS CERASUS).

Cherries should be brought to the factory in small boxes just as they are handled for the retail trade. They should be stemmed and then washed. The California fruit is graded for size over screens having openings of twenty-two, twenty-four, twenty-six, twenty-eight, and thirty-two thirty-seconds of an inch. The cherries may or may not be pitted, but generally it is preferable that this be done. The new machines do the pitting rapidly and well. The cherry rests in a cup-shaped opening and the seed is forced out by a small cross-shaped plunger. There is naturally some lacerating of the flesh, but not more than is usual in the pitting by hand. After the cherries have been pitted they should not be permitted to accumulate in masses of more than 2 inches in depth. The quantity should be weighed for each can and a heavy sirup added, or they should be heated in a preserve kettle and filled in the cans hot. The latter method gives a better fill, but breaks or tears the fruit to a greater extent. The enameled can is preferable for this fruit. The process is 18 minutes for a No. 2½ can. White cherries are usually canned without pitting, and in a lighter sirup than is used upon the red.

GOOSEBERRIES (RUBUS GROSSULARIA).

Few gooseberries are canned, and these are largely used for pies. The berries are gathered when nearly ripe and are handled in baskets and shallow boxes. The first operation at the factory is to remove the stems and brown blossom ends. This was done formerly by running them over a vibrating screen upon which was directed a strong blast of air. This removed part of the blossoms and stems, and the remainder were either rubbed off by hand or were passed with the fruit. An improved gooseberry cleaner consists of a slitted disk, below which parallel knives revolve. The berries are poured above the disk and made to roll over and over by light dragging chains. This causes the stem or blossom to fall into the slits, where they are cut off close to the berry. The berries are then washed and filled into cans by weight. Those intended for pie making usually have only water added, while those for the general trade have a sirup. The filling, exhausting, and capping are the same as for other berries.

GRAPES (VITIS VINIFERA).

Grapes have not been used very extensively for canning purposes, but there has been a noticeable increase in the past few years. In the East the white variety is used almost exclusively, but on the west coast both the white and the colored grapes are canned. They are gathered when the flavor is fully developed, but the fruit is firm. The bunches are hand picked, washed, and put in cans to within one-fourth of an inch from the top. A hot sirup is added, the cans are exhausted, and then closed. The process is about 14 minutes at 212° F. for a No. 2½ can. In California the grapes are also graded by size, being run over screens having holes twenty, twenty-two, twenty-four, and twenty-six thirty-seconds of an inch in diameter. The sizes thus separated are not indicated on the label and the consumer is unaware of this refinement except as it is indicated by the price.

PEACHES (PRUNUS PERSICA).

The peach is one of the most popular fruits canned and the quantity so used is enormous. It leads all other fruits in value. The principal packing is done in California, New York, and Michigan. In California the lemon cling, or some one of its varieties, is the favorite, while in New York and Michigan the freestone variety is preferred. The growing, picking, and handling are the same as for the market; that is, they are hand picked just before turning soft and handled in crates or baskets.

The conditions for growing peaches are so favorable in California and they acquire such size that they are purchased on the basis of being 2¼ inches or more in diameter, those below that size being received at a reduced price. The eastern packers can not make such close discrimination. If the peaches are well ripened, they are run through the factory at once. If they are under ripe, or hard, they may be kept in a cool place for a time. It is the practice of some to pick the peaches over each day and take them out when at their best, but as a rule they are held until all can be used at the same operation. Holding the fruit in cold storage is not advisable, as the flavor is impaired. Some varieties of peaches are graded for size before peeling, and this is done on an orange grader, the space between the rolls being adjusted to deliver three, four, or five sizes as desired. When the lye-peeling system is used the grading is generally done after peeling. The holes in the screen are sixty-four, sixty-eight, seventy-two, and seventy-six thirty-seconds of an inch in diameter, which, with those that will not go through the largest opening, gives five sizes. Careful grading for size is almost wholly limited to the California product.

There are three methods of peeling: By hand, with the knife; by steaming and slipping the skin; and by the use of lye. There have been some machines devised for peeling, but they have been used but little. The knife used for hand peeling is provided with a somewhat curved blade and a guard to limit the depth of the cut. This is the method used almost exclusively in the East and on a portion of the California pack. After the peach is peeled it is split along the line of natural cleavage and the pit forced out in the freestone or removed with a pit spoon in the cling varieties.

Peeling by means of steam is possible with only a few varieties. This method consists in splitting the peach, removing the pit, and placing the halves in a single layer, split side down, in a tray which has a covering of cheesecloth. When the tray is covered, the cheesecloth is folded over the peaches and the tray slipped into a steam box. The peaches are heated in this way for about 3 minutes and then the skin may be slipped by picking it up between the fingers. This method involves very careful work, but results in a handsome product.

When the lye system is used, the peaches are first split and pitted and the halves placed in special machines containing hot, weak solutions of caustic soda or lye. They are carried through just fast enough to allow the peel to be removed, the time usually being 12 to 18 seconds. As soon as they emerge from the soda solution they are thoroughly sprayed and are kept under sprays or in water until they are placed in the can. Where the lye peelers are installed, the peaches are usually delivered to the filling tables, graded into sizes, as already indicated, but the fillers sort for quality, separating the pieces perfectly ripened, those unevenly ripened or defective in color, and those imperfect in form or in quality.

With these different grades 60°, 50°, 40°, 30°, and 20° sirups are used. In addition peaches are canned in slices, and while formerly it was the practice to use the imperfect halves and small sizes for this class, now equally as good stock is used and siruped in the same way as the halves. There is a grade of water or pie peaches made from the lower grade stock. The cans are filled by weight.

One of the best factories using the steaming system to loosen the skins, placed the peeled peaches on pie plates and weighed the quantity necessary to fill each can. The plate was washed every time it was used.

Hot sirup of the degree desired is added to each can until it is full. It is exhausted for 3 minutes and processed for 25 minutes at boiling temperature.

PEARS (PYRUS COMMUNIS).

Pears used in canning are grouped generally in two classes, hard and soft, the former being represented by the Kiefer and the latter by the Bartlett. While the Kiefer yields very well, the consumption is small, largely because of the poor quality; the Bartlett is much better, but often the label does not adequately tell the story.

The canning of pears is similar to that of canning apples. The work of peeling, coring, and halving, however, is done by hand. Considerable care is taken in trimming to a symmetrical form and in removing the core to cut away only so much as may be necessary to remove all trace of seed cells. They are graded in three classes, dependent upon size or number of pieces required to fill a can, and uniformity of shape and texture. This is done according to the judgment of the filler and not by machine. They are put into cans the same as apples and a sirup is generally used instead of hot water, as it retains the flavor much better. The process is 16 minutes at 212° F. for No. 2½ cans.

PLUMS (PRUNUS DOMESTICA).

The classes of plums are generally quoted on the market as green gage, yellow egg, and Lombard. Other varieties are used, but these are the popular ones. The plums are selected when just ripening. On the Pacific coast they are graded for size by running them over screens having openings thirty-two, forty, forty-eight, and fifty-six thirty-seconds of an inch in diameter. They are washed, put in cans with sirup, and processed 14 minutes at 212° F. for a No. 2½ can.

RASPBERRIES (RUBUS OCCIDENTALIS AND R. IDAENS).

Raspberries are grown and harvested the same as for the market and should be delivered to the factory in berry boxes. At the factory they are handled in exactly the same manner as are strawberries.

Red raspberries and black caps should be kept separate. Columbia berries are regarded as inferior in appearance to Cuthberts, being less bright in color, but they have excellent flavor. The use of a sirup of the right density has much to do in bringing out the full flavor. The cans should be enameled-lined to retain both color and flavor. The process is 12 minutes at 212° F.

STRAWBERRIES (FRAGARIA VIRGINIANA).

Strawberries used for canning are grown the same as for market, and such varieties as are firm and of uniform size should be used. They should have a well-developed flavor, a little more acid than is desired for eating raw. They should be gathered the same as for the market, in boxes holding not more than 1 quart and preferably only 1 pint, the object being to deliver them in the best possible condition, without bruising or mashing.

A distinctive method of handling berries on the Pacific coast is in a chest of shallow crates. These chests are well made and hold four tiers of five trays each. Each tray measures about 8 inches wide, 15 inches long, and 1½ inches deep inside. The boxes holding the berries are therefore very shallow and there may be two or more in a tray. The fruit arrives at the factory or market with the minimum of bruising.

On arrival at the factory different methods are followed, but one of the best, as practiced by one of the large packers, is as follows: The boxes are delivered to tables, where they are turned out upon enamel pie plates. The berries are stemmed, defective ones sorted out, and any foreign substance removed. The plate containing the berries from a single box is passed to another helper, who washes the fruit under a spray; the next one weighs each plate and adds the correct amount to fill one can. The berries are poured from the plate into the can, in which operation a special half funnel is sometimes used. The can should be filled a little above the level. Hot sirup is added and the can given a 2-minute exhaust, sealed, and processed for 14 minutes at 212° F. The cans should be preferably enamel lined, with open tops.

In the handling of the fruit at this plant the pans are washed after each separate usage. The work involved is greater than in some other systems, but the product can hardly be excelled in cleanliness and in flavor.

At some other plants the berries are stemmed from the baskets and are run through a fruit washer to remove any leaves or dirt; they are then filled directly into cans without weighing or are collected in large pans, and when a sufficient quantity has accumulated are then put into a preserving kettle with sugar and heated until they just come to a boil. The berries and their sirup are then filled into the cans.

Strawberries do not admit of being handled by automatic machinery. The stemming must be done by hand, but in the plate system they are not touched after once being washed. In the system in which considerable dependence is placed upon the fruit washer the cleaning is well done, and in a manner not to injure or break the fruit. Cans which are well filled with cold fruit will not be full of fruit after processing. The heat causes the breaking down of the tissue and consequent loss of juice, so that the berries will float. Berries heated with sugar in the preserve kettle will give a better fill in the can, as more juice is cooked out than can be returned to fill the space between the solids. In this practice there is a distinct difference between the manufacturer who attempts to give a can with the maximum of food solids and the one who cooks the berries to abstract the juice for other use, such as fruit sirups for soft drinks. A sirup should be used in all cans, as it holds the flavor much better than water. Sugar is always used with such fruit, and the proper time for its application is when it is being cooked. The degree or density of the sirup is a matter of taste, but preferably it should be fairly heavy. The enamel-lined can is decidedly the best for preserving flavor and color, and also for resisting the action of the fruit on the can. Strawberries are also put up in glass and given the same general treatment.

VEGETABLES.

ASPARAGUS (ASPARAGUS OFFICINALIS).

Almost the entire asparagus pack of the United States is put up in California. The asparagus beds are located on the bottom lands which have been reclaimed from the Sacramento and San Joaquin Rivers and are exceedingly rich. Dykes have been constructed, canals dug, and pumping machinery installed, so that it is possible for the growers to control the conditions to such a degree as to produce enormous yields of a very high quality. The advantage is so great that other points can not compete successfully.

The asparagus is grown in large fields in rows, and in the fall the plants are cut, the tops acting as a mulch. The earth is banked over the rows to the depth of a foot or more. In the spring the stalks come through this light soil and mulch and are practically bleached. They are cut every day or every other day, the stalks being selected just as they appear through the ground. The work of cutting must all be done by hand by means of a long chisel-like knife, and is very laborious. The object is to cut the stalk back 7 inches or more. The asparagus is collected in hampers or crates and hauled promptly to the cannery. It is essential that the work be done promptly to insure a crisp article. A delay will cause a fine product to become tough and stringy. At the factory the first operation is to turn the crate upon a sorting table, where the stalks are sorted into five grades, based on size, also into two qualities, dependent upon whether the stalks are wholly blanched or partially green. A further sorting is made, dependent upon whether the stems are straight or crooked. All the sorting is done by hand. The five grades for size are known as giant, mammoth, large, medium, and small, and these are based upon the number of stalks which will go into a standard No. 2½ square can. With giant stalks about 14 are required; mammoth, 20 to 22; large, 30 to 33; medium, 40; and small, 50. What are known as asparagus tips are put up in cans just one-half the regular size, and about 30 per cent more stalks are required to fill the can. What are known as hotel tips are the cuttings made in trimming the asparagus to size, and the whole stalks which are crooked or deformed. The quality of these is just as good as the other, though not so pleasing in appearance. Some of the large asparagus is peeled, or stripped, as the operation is more properly called.

After the grading the tips or stalks are cut in lengths to fit the can, and then thoroughly washed in cold water. They are next blanched in wire baskets, the stalks being held in position so that they will not move about. The length of the blanch depends upon the condition of the stalks, being a mere dip in hot water in some cases, and as long as 3 minutes in case of advanced growth. On coming out of the blanch they are dipped in or sprayed with cold water to prevent softening, after which the cans are filled immediately. A light brine is used to fill the interspaces, the can exhausted, the cap placed on, and a process of 240° F. given for 12 minutes. The cans must be well cooled at once.

A great deal of fancy is shown by the consuming public in buying asparagus. The absolutely white is demanded, and brings a premium of from 25 to 50 per cent in the market. The green is just as good and in many instances better, though it does not look quite as attractive, and the liquor is likely to have a more or less cloudy appearance, due in part to the breaking of tips and side buds.

BEANS, GREEN (PHASEOLUS NANUS).

String beans form a regular side dish at almost every hotel, and they are generally the canned article. There is a large pack of beans put up each year, and while hotels and restaurants were formerly the principal buyers, a large demand for home use has been created in the past few years. The beans raised for canning are produced the same as for the market. The growth is best when the season is fairly moist and cool, the majority being produced in northern New York and Michigan, and more recently large packs have been put up in Wisconsin.

The beans are picked by hand and the object is to gather them as young as possible. The best are about 2½ inches long and less than a fourth of an inch in thickness; the large beans become tough and stringy. At the factory the beans are graded in five sizes by means of special machinery, the essential feature of which is a series of vibrating screens made of rods or bars running in one direction. These rods are generally set eighteen, fourteen, eleven, and eight sixty-fourths of an inch apart. The beans are fed in over the coarser screen first and those which fail to pass through constitute one grade, and as they pass to each succeeding screen the next larger sizes are separated and the smallest pass through the last. The work is done better than was formerly done by hand.

The next step is to snip or string the beans. Some varieties of beans are so nearly stringless that the simple snipping of the ends is sufficient, but when they become old, hand stringing is necessary. The cutting of the ends, or snipping as it is called, can be done well by machinery. It is also the practice to cut the large beans in lengths of about 1 inch. All beans are well washed, placed in wire baskets and blanched, or they may be blanched in the cylinders used for peas. The time required for blanching will vary with the age; the small size of young beans will require only about 1½ minutes, the larger ones if tender will require about 4 minutes, and if hard and tough they may require 8 or 9 minutes. It is the rule of good processors to blanch until the beans are tender, irrespective of time, and for that reason many prefer the basket in a tank of boiling water to the pea blancher.

The blanched beans are filled into the can by means of a special bean filler. This machine carries a tray, holding 4 dozen cans, and has a hopper above it with holes corresponding to each can. The beans are poured into the hopper, the quick vibrating motion of which shakes the beans into the can. As a further precaution against short weight, each can is weighed and any deficiency in fill is made up by hand. A weak hot salt brine is used to fill the interspaces in the cans, which are exhausted, capped, and processed for 30 minutes at 240° F.
as for peas. A full can should weigh not less than 13 ounces, exclusive of the liquor.

BEANS, LIMA (PHASEOLUS LUNATUS).

Lima beans are grown for canning both as a green bean and as the bean in succotash. There are two varieties, the pale or true Lima and the bush variety. The former is but little grown for canning, as it must be gathered by hand the same as string beans, while in the case of the bush beans the whole vine is taken up and hauled to the factory, as in the case of pea vines, and then run through a pea viner to shell the beans. The speed of the viner is changed to meet the altered conditions. The beans are graded generally into four sizes, if canned, but are left ungraded if intended for succotash. It is also becoming the custom, as with peas, to can some beans ungraded. A better flavor seems to result from the combination than is found when they are canned separately. The sizes are as follows, and are obtained by sifting over the screens with openings twenty-four, thirty, thirty-one, and thirty-two thirty-seconds of an inch. Those passing through the first screen are called tiny; through the second screen, fancy; through the third screen, medium; through the fourth, standard. Those passing over the last screen are sometimes designated large or mammonth beans. The beans are blanched the same as peas, and the can filled, so that after processing it will be full and just covered with brine. The process is the same as for peas. A full can should weigh not less than 13 ounces, exclusive of the liquor.

BEANS, WAX.

Wax beans are handled in the same way as string beans. More attention, however, is paid to sorting, as any spot will show on the light surface. The weight of the beans in the can should be not less than 10 ounces, exclusive of the liquor.

BEETS (BETA VULGARIS).

Beets grown for canning must be of a deep-red variety, evenly colored throughout. Pale or uneven colored beets present a very poor appearance in the can. The beets used for canning are mostly grown in New York, and are cultivated the same as for the garden, but in large acreage. The tops are cut off and they are hauled to the factory as are tomatoes. The time of packing is in the fall, usually the latter part of September.

At the factory the beets are graded into four sizes—small, sometimes called rosebud, the beet being less than 1 inch in diameter; medium, the beets being from 1 to 1½ inches in diameter; large, those from 1½ to 2 inches; and very large, those over 2 inches. The very large beets must be cut into pieces for canning, and for that reason are called cut beets. The grading is done in a wooden squirrel cage having the slats set at proper distances or over tables having holes of the size indicated.

After being graded the beets are soaked in tanks of water to soften the adherent dirt and then sprayed well. The beets are next placed in large iron crates or heavy iron baskets, placed in the retort, and steamed for 20 minutes at 220° F. This loosens the skin so that they may be peeled with the best possible results. The peeling is done by hand, as is also the filling of the cans. Only water is used on the beets, though salt may be added at the rate of a teaspoonful to the can; enamel cans should be used, otherwise the beets will be discolored. The process on beets is 245° F. for 1 hour.

CORN, SWEET (ZEA MAYS).

Canned corn is the result of the persistence of Isaac Winslow, of Maine. He was a sailor by occupation, and in his wanderings upon the high seas visited France and learned of the method of preserving food by canning. The advantage of such foods, particularly to sailors, was obvious. Mr. Winslow began experimenting on the canning of corn in 1839, the first trials consisting in boiling the corn on the kitchen stove for varying periods of time. The cans were marked and a record kept of each lot. The results were mostly failures, but a sufficient number of cans were saved, and these were of such good quality that the efforts were continued. The succeeding years gave essentially the same result. In 1843 he built a small boiler to generate steam and a wooden box in which to put the cans, so that the cooking might be done in a closed steam chamber. As the results were less successful than in the previous years, the steam box was discarded. It was not until 1853 that he had sufficient success to warrant applying for a patent on his method, and it was regarded with so much distrust that the letters were not granted until 1862. Winslow first packed the corn on the cob, but this was bulky, and he believed that the cob absorbed some of the sweetness. He next pulled the kernels off the cob with a fork, and finally cut the corn with a case knife. Winslow's apparatus and methods were crude, but he discovered the principles which underlie the canning of corn. It may also be said that he and his successors brought fame to Maine corn as a canned product, and this reputation persists to the present time.

The canning of corn is a large industry in Maine and other States extending from New York to Maryland, west to Iowa, and north to Minnesota. In most of the Eastern States the crop is grown by numerous farmers in small patches of a few acres, while several of the western factories raise their own corn, covering hundreds of acres. At Hoopeston, Ill., two canneries use the product of 7,500 acres. Claims are made that certain sections produce better and sweeter corn than others. This is not always sustained by facts, for quality is also affected by the variety and state of maturity when gathered. Again, some canners pay more attention to the quantity of corn grown on an acre than to the quality. The seed used is grown by specialists, as a rule, and a very large part of it comes from Connecticut, a State in which no canning of corn is done. The type of corn used now is quite different from that canned several years ago. The effort is to develop a tender, fine-flavored sweet corn. The ears are of two types, those having large, flat kernels arranged in rows and those with small, long kernels irregularly placed. Stowell's Evergreen is typical of the former type and Country Gentleman of the latter. The corn is planted and cultivated like field corn, and is gathered by snapping off the ear when it is in its prime. The ears are hauled to the factory in the husk in order to protect the kernels from injury in handling and from dirt and exposure.

A modern corn-canning plant is a large establishment, equipped with valuable automatic machinery to do the work in a rapid, cleanly manner. When the corn arrives at the factory it is dumped from the wagon onto a conveyer, which carries the ears to different parts of the husking shed as they are needed. Most of the husking is done by hand, but this will undoubtedly give way to machine methods, as the husking machines have been almost perfected in the past year. As rapidly as a bushel measure is husked it is put upon a conveyer, and while on the way to the silking machine is sorted for quality. A high grade can be secured only by selecting ears with grains which are uniformly tender. Corn which is too old or too young to make a fancy grade of goods is taken out and held until a sufficient quantity accumulates to make a run on a lower grade. The silking is done by means of rapidly revolving rolls and brushes. As the ear revolves on its axis and at the same time is carried forward, it is gently wiped by rapidly revolving brushes, which pick up any silk that may be attached. This work is done with remarkable rapidity and by machinery so carefully adjusted for any irregularity in the size of the ears or even in the same ear that there is no chafing or bruising of the tenderest grains. This process is immediately followed at some factories by a thorough spraying with water, while at others this is omitted, the claim being made that a certain flavor is lost.

The corn is cut by machinery, and from the time the ear is fed into the cutter until the corn is sealed in the can it is not again touched by hand. The ear is forced through a series of curved knives, mounted in an adjustable circular frame, so that they will accommodate themselves to the varying size of the cob. Scrapers complete the work by removing the grain and soft bits of kernel at the base. The corn again passes through a machine to remove bits of silk, husk, or cob, so that the final product is as clean as labor can make it. This cleaner consists of a series of wire combs, which intermesh as the corn passes through, and wire cylinders which act as sifters.

The corn is next mixed and cooked, and in this operation it is necessary to add some water, otherwise it would become a dry, tough mass in the can. The quantity of water used will depend upon the consistency desired and the condition of the corn. Some varieties require more than others, but the average quantity used in cream corn is about 5 ounces per can. It is also usual to add both salt and sugar to the corn to give the desired flavor. This is used in all grades, though more carefully in the high grades than in the low. The eastern packers, as a rule, use more sugar than the western.

The care with which the cooking is done before the corn enters the can determines in a large measure its appearance. The addition of too much brine will give a sloppy can, while the use of too little gives a dry can. Insufficient cooking will leave the brine and corn separated; the quantity of brine may be right but the corn may be dry in the bottom of the can and most of the brine on top, or they may be mixed but not blended. The preliminary heating is done by steam, using automatic machinery, which heats and evenly mixes the corn and brine and at the same time fills the cans. The corn enters the cans at about 180° F., and the capping is done in the usual manner.

Corn is one of the most difficult products to process. It requires a temperature of about 250° F. for 75 minutes to insure sterilization. There are packers who process at from 240° to 245° for 90 minutes, and others who process their corn twice to insure keeping. The higher the temperature the browner the corn and the more pronounced the cooked taste. The consistency of the corn makes a great difference in the heat which must be given; the drier the corn the slower the heat penetration.

Corn is packed as "cream corn," or, as it is sometimes called, "Maine style," the kernels being cut as already described and the portion scraped from the cob added. The product should be of a thick, creamy consistency. Again the corn is cut from the cob as closely as possible by knives, but only the whole grains are used, the bits and scrapings being discarded; corn used in this way must have long, slender grains, commonly called "shoe peg," and the quantity of brine be such as to keep the kernels separate. This method of preparation is called "Maryland style" by the trade. In some instances the corn is run through a recutter, which gives a grainy effect or one like the cream corn, depending upon the method of handling. This procedure is also followed in working up corn which has become too old to make a good regular pack. Corn may be run through slitting machines, which cut the grains open on the end and then squeeze out the contents, leaving it free from hull. Cut corn is also run through a "cyclone," a machine for forcing the creamy portion of the kernel through a fine sieve, thus removing all of the hull and giving much the appearance of green corn meal.

Field corn is not used in canning. Some of the sweet corn used produces very large ears and coarse grains, which give rise to the suspicion that field corn has been substituted. There has been a very general improvement in sweet corn in the past 10 years, and it will probably not be long before this coarser variety will give way to a better and sweeter one.

A can of fancy corn upon opening should be well filled (within three-eighths of an inch of the top), should be absolutely young and tender stock, medium moist, practically free from silk or bits of cob or husk, only slightly darker than natural or of a light golden-brown color, and have the distinctive young corn flavor. The weight of the contents should be about 21 ounces. If put up in "Maryland style," the kernels should be separate and the brine nearly clear and the corn should weigh not less than 13.5 ounces, exclusive of the liquor.

A can of standard corn should be well filled, reasonably tender, fairly bright color or slightly brown, and nearly free from silk, bits of cob, or husk. The flavor should be characteristic of young sweet corn. If put up in "Maryland style," a part of the kernels may be somewhat hardened and the brine a little cloudy.

PEAS (PISUM SATIVUM).

The transition from growing a small patch of peas in the garden to supply a few meals of a choice vegetable during the growing season to that of growing hundreds of acres to supply a canning factory packing an article available at all seasons is but an incident in the development of a great industry. The garden bed was spaded, raked, and planted by hand. Brush was obtained from the orchard or wood lot, and the rows "stuck" in order to insure the vines proper support. When the green peas were picked and carefully prepared, they made what was at one time styled a dainty dish. The fields are now cultivated, sowed, and the crop harvested by machinery the same as any farm crop. There are several factories which take the entire yield from more than 1,000 acres. The plants selected have sufficient rigidity, no added support being necessary. The whole plant is hauled to the factory while fresh and green, the same as a load of hay.

The canning of peas dates back to the beginning of canning, and is one of the three large crops packed. In this country it is confined largely to those States having a cool spring and plentiful rainfall. The southern limit of successful growing seems to be from Maryland west to Indiana and northwest to Minnesota. Some peas are also grown on the highlands in Colorado and a few on the western coast. Wisconsin, Michigan, New York, and Indiana lead in this crop.

The pea used for canning belongs to the garden variety, *Pisum sativum*, of which there are two general classes, early, or round smooth pea, and the wrinkled pea. The latter are much the sweeter. The Little Gem and Alaska are typical of the first class, and Horseford's Market Garden, Admiral, and Advancers of the second.

The peas are generally sown upon good ground, well prepared, as early in the spring as frost will permit, and no subsequent cultivation given (except in California). Instead of all being sown at one time, the seeding is made to extend over several weeks, in order to prevent too many maturing at one time. When the peas are well grown and are still very tender, they are cut by mowing machines or special pea harvesters, and are then loaded upon wagons and hauled to the factory. Until a few years ago, the pods were picked from the vines in the field and taken to the factory in baskets or bags. This necessitated a very large force of men, women, and children in harvesting, and added much to the cost of the product. There are only a few factories in the United States which follow this method at the present time, and it is limited to a part of the pack.

The vining machine, which is used for separating the peas from the pods while they are still on the vine, is a very simple and ingenious device to accomplish a difficult task—the shelling of the tender pea so carefully that it will not be injured. It consists of a large cylinder, perforated with many holes, which are large enough to permit the peas to pass through, but not the vine. Within the cylinder is a heavy shaft, bearing strong paddles or beaters. The cylinder is made to revolve rather slowly and the beaters very rapidly, in the opposite direction. The vines are fed in at one end of the cylinder, are carried upward by its motion, and fall upon the beaters, which strike the pods, causing them to burst open and discharge the peas. The peas roll out through the holes in the cylinder, and the vines pass out the opposite end. The present vining machine is a modification of the podding machine which was invented by Madam Faure. It was the first important step in the development of the pea-canning industry.

The next step in the process is that of cleaning, and it consists of two operations; first, that of passing the peas through a fanning mill to remove pieces of pods, leaves, and dirt, and second, in washing, which is done in wire cylinders known as squirrel cages. These cylinders are set on a slight incline and made to revolve slowly, so that peas which enter at one end gradually roll out the opposite end, and while doing so they are well sprayed with pure cold water. After the washing, the peas are graded for size. This is done by passing them over vibrating screens, which have holes of a definite size, or through cylinders, with sections having perforations corresponding to those in the screens. The perforations are standard and give the following sizes in the peas: Petit pois, extra sifted, sifted, Early June, marrowfat, and, in the case of late peas, the telephone. If the peas are properly labeled, they should be uniform in size. Some manufacturers, instead of turning out all these sizes, combine two sizes in one. A few peas are sold ungraded or with only the first and second size taken out. The petits pois should pass through an eighteen sixty-fourths inch hole; the extra sifted, or extra fine, through a twenty sixty-fourths inch hole; the sifted, or fine, through a twenty-two sixty-fourths inch hole; Early June, through a twenty-four sixty-fourths inch hole; while the marrowfats pass over the ends of the screens. With sweet wrinkled peas, a twenty-six sixty-fourths inch screen is used to separate the marrowfats and those remaining above pass over as telephone size. These designations, which were partially adopted from the French, have been in use for a long time, and refer to size and not to variety nor to time of gathering, as would be inferred from the name "Early June." The term "Early June" has, in recent years, come to have another meaning, that of including all of the smooth or Alaska group of peas in distinction from the sweet wrinkled varieties. We therefore find smallest-sifted Early June, extra-sifted Early June, and sifted Early June, as distinguished from the same names applied to sweets. The trade terms have little meaning to the consumer and could be supplanted by proper descriptive terms to the advantage of all concerned.

Peas are also graded for quality, those being small, young, and tender, so they will crush easily between the thumb and finger are considered to be the highest grade, while those which have a considerable percentage hard, turn brown upon processing, or cause clouded liquor in the can, are of a lower grade. The grading is done largely upon the judgment of the inspector as the peas arrive, and later by the superintendent.

The peas may be mechanically graded for quality before, but preferably after, grading for size. This is done on the basis that the old or hard peas are heavier than the younger and more tender ones. Peas will not all mature alike on the same vine, nor in the same field, so that it is not possible to cut them to secure absolute uniformity. The more slowly the peas mature under fairly cool moist conditions, the tenderer they will be, so that in some sections the necessity for grading for quality is less than in others. This grading is effected by means of brine, which is made to a strength that will float those that are tender, the harder ones sinking. The first quality can be skimmed off, and those that sink again separated in another and heavier solution, giving a second and third grade. The first grade will be lighter in color, softer on pressure, and give a clear liquor on canning; the second grade will be slightly darker, and the liquor cloudy; while in the third grade, the size will be uneven, the peas dark and hard, and the liquor very cloudy and thick. In dry seasons the grading will not be so good as there is less difference in the weight of the peas. It is possible to get 15 grades of peas, depending upon size and quality, from the same load, the difference being sufficient to be easily distinguishable in the finished product.

When the peas leave the graders they pass over slowly moving belts in a single layer, and those which are split, off color, or defective are picked out. This is the only operation in which it is necessary to touch the peas with the hands.

The peas are blanched, or more properly parboiled. They are boiled just long enough to soften them uniformly and to remove the mucous substance on the outside. The time for the blanching will vary from one-half minute for the very tender small peas, to 15 minutes for the overmatured large ones, some variation being necessary for each size and degree of hardness. Most of the blanching requires from 1 to 4 minutes.

The matter of blanching is exceedingly important, for upon it depends in a large degree the appearance of the peas and the character of the liquor. There are several different styles of apparatus in use for blanching, the simplest being a large trough containing scalding water in which wire baskets containing the peas are placed for the required time. Another device consists of a cylinder which is made to revolve in a tank of water and gradually cause the peas to pass through in a continuous stream by means of a large screwlike conveyer. The latest type is a tank having three compartments; the peas are fed in at one end and the hot water at the other, so that the water in which the peas are first scalded is being constantly renewed from the next tank, and, as the peas emerge, they come from the freshest, cleanest bath. The peas are again washed after blanching and before going to the filling machines.

The pea fillers should measure out a given quantity of peas and deliver it into the can with the minimum of cutting or bruising. The greater the number of injured peas the less attractive the contents, both because of splits and cloudy liquor. The fillers should be adjustable that the cans may receive a fill according to size and age. The younger and smaller the peas the greater the fill, and vice versa. Old peas absorb liquor in the process, while the succulent ones take up very little. The liquor used in canning peas is made up of water, salt, and sugar, the proportions being a matter of taste. The eastern packers, as a rule, use more seasoning than the western. The liquor is added after the peas have been put in the can. The subsequent capping and processing is the same as for corn. The process is from 235° to 240° F. for from 35 to 40 minutes, depending upon the freshness and state of maturity. The cans of peas should be given a cold bath at once after the process is finished, in order to arrest cooking and insure a clear liquor.

The canning of peas requires special care. If a fine product is to be secured, there must be careful selection in the field and continuous and rapid work from start to finish after the vines are cut. "Only an hour from the field to the can" is not literally true, but it is approximately so. The work is almost wholly done by automatic machinery, connected by special conveyers in such manner as to insure continuous action. At all the various steps the washing is of the most thorough character, and in some of the best factories almost a gallon of water is used in the preparation of each can. The highest grade of American peas represents the best that is accomplished in the pea-canning industry, and are unexcelled by any foreign production.

The cost of a can of peas will vary with the size and quality. The very tender smallest sifting peas, or "petits pois," are the most expensive for the reason that but comparatively few are produced; not more than 5 per cent of a good crop will be of that grade. The price gradually decreases through the sizes to the marrowfat, which is the cheapest. There is more nutrition in the larger sizes and, if properly graded, they have the better flavor. Ungraded peas have a particularly good flavor, though they are not so attractive because of lack of uniformity.

A well-filled No. 2 can of peas should have a net weight of about 21.5 ounces, of which slightly more than 14 ounces should be peas and 7 ounces liquor.

A can of first-grade peas should be from selected field stock, or the lightest weight if separated, and the can should be well filled with peas that are uniform and true to the size indicated, even in color, absolutely tender, of good flavor, and covered with a clear liquor. The weight of the peas, exclusive of the liquor, should be not less than 12.5 ounces.

A can of standard peas should be well filled with good field-run stock, the peas fairly uniform, of the size indicated, and covered with liquor, which may be more or less cloudy but not thick. There may be some variation in color, but the peas should be tender, or only a small proportion hard, and of good flavor.

PUMPKIN (CUCURBITA PEPO L.).

It used to be the custom to associate pumpkin pie with the Thanksgiving season, but the tin can has lengthened its season to the full year, and made it especially convenient for the home piemaker.

The pumpkins used for canning should be of a hard, sweet variety, and evenly ripened. The meat should be of good texture, golden yellow, but not watery. It has been the custom generally to grow the pumpkins with the corn, but a few canners find that a more satisfactory yield and a far more uniform quality are obtained by growing in the open field as a special crop.

The pumpkins are carefully selected, stemmed, and well washed to remove any adherent dirt. They are cut into large pieces, either by knives or roller disks, and are given a general washing in a heavy squirrel cage, the principal object being to remove the seeds and loose fiber. The pumpkin is then put into large iron crates and cooked in the retort until it softens, which requires about 20 minutes at 240° F.; it is next run through a cyclone, which removes the hard part of the skin and the tough fiber. The pulp proper is cooked very little if it is of a good consistency, but if light or thin it is evaporated until it is of the right body. It is filled into cans while hot, sealed at once, and processed at 250° F. for 90 minutes.

Some packers cut the pumpkins in halves and peel and core with special revolving knives. This necessitates considerable extra hand work, but is particularly advantageous when the pumpkins do not ripen uniformly. It does not have any apparent advantage over the direct-heating method if the raw material is of uniformly good quality.

Pumpkin is packed almost exclusively in No. 3 cans, which should be enamel lined, thus preventing action on the tin, and also aiding in the retention of better color and flavor.

A good can of pumpkin when opened should be filled within one-half inch of the top; should be fairly heavy, smooth, evenly screened, free from fiber, and uniformly colored. A can lacking an inch or more of being full, coarse, containing fiber, or being thin and watery, is not a first-class article and is short weight. A No. 3 can should contain at least 32 ounces. Squash (*Cucurbita ovifera*) is grown and handled the same as pumpkin.

RHUBARB (RHEUM RHAPONTICUM).

Rhubarb is grown in fields, in rows 4 feet apart and hills about 2 feet apart in the rows, and is cultivated the same as are potatoes. The soil must be rich to give a luxuriant growth. It is harvested when the leaf stems are of large size, which may be at any time from the middle of May until the middle of August.

In harvesting the best stalks are selected, the small or undesirable ones being left to take care of the plant. The pulled stalks are made into bundles; the leaf and butt are then cut off and the stems placed in crates to be hauled to the factory. The hauling is done the same as in the case of tomatoes.

At the factory the rhubarb is washed in large tanks of running water and at the same time inspected for any imperfections. The next step is the cutting, and this is accomplished by means of a series of small saws set 1 inch apart on a shaft. The rhubarb is laid on a carrier, which feeds each stalk crosswise to the saw. The pieces ready for the can are therefore 1 inch in length and the size of the stem. The cans are filled with a string-bean filler, and as much is put in as can be shaken below the level of the rim. Hot water is added to fill the interspaces.

The practice in some factories differs in some particulars from that given here. First, in that the stems are stripped or peeled before being cut, and, second, in that the rhubarb is heated in a preserve kettle before filling into the can. In the latter case only a very small quantity of water is used, as in the cooking sufficient juice is extracted to furnish part of the liquor in packing. This style of pack is put up in No. 3 and No. 10 cans. The former is put up only in No. 10 cans for pie purposes. The process is 13 minutes at boiling temperature.

SUCCOTASH.

Succotash is a mixture of green corn and green beans, the Lima bean being the one generally used. Succotash has also been made from green corn and soaked beans, as in most places the corn and beans will not come to maturity at the same time. The flavor of succotash made from good corn and strictly green beans is better or more delicate than that made with dried beans; otherwise the latter is in no way inferior to that made from the green bean; but when the dried bean is used the fact should be indicated on the label. In the regular field run of Lima beans some will be further advanced than others; while the pods may all be green, in blanching some of the beans may turn white and on breaking they may appear mealy, and thus give the appearance of being soaked when the can is opened. In fancy succotash these white beans are picked out by hand. A succotash should consist of not less than 20 per cent of beans, and in the high grades there is more nearly 40 per cent beans, either graded or ungraded for size. The cut corn and blanched beans are mixed, after which they are treated the same as corn, being given the same sugar and salt brine, preliminary cooking, and process. The net weight in a No. 2 can should be not less than 19 ounces.

SWEET POTATOES.

Sweet potatoes can be canned to good advantage for use in those sections where they can not be raised. They usually come under two classes, the long yellow variety, growing extensively in New Jersey, and the light or southern variety. The former is preferred in the market at present, partly because of its better appearance. The southern variety turns dark and may become watery.

The potatoes are washed and the skins removed by running them through a potato parer or abrading machine such as is used in hotels, by dipping in a hot, weak lye, or by boiling until the skin can be scraped off easily. If they are not large in diameter, the cooking may be continued until they are tender to the center or two-thirds cooked. If they are large, it may be better to cook first until the skin can be removed and then give a second cooking, either in boiling water or in a steam retort, until they are about three-fourths done. They are packed in the cans as closely as possible without mashing and preferably without the addition of water. They may also be grated or mashed, and in this form are offered for sweet-potato pies. They are then thoroughly exhausted and processed for 70 minutes at 240° F. or for 3 hours in boiling water.

TOMATOES (LYCOPERSICUM ESCULENTUM).

The time is easily within the memory of many persons when tomatoes were thought to be poisonous. A few persons in the Eastern States used them 70 years ago, but they did not become common until a much later period. In the West the prejudice against them persisted until less than 40 years ago. The first record of canning tomatoes is that of the work done by Harrison W. Chrosby in 1847 at Jamesburg, N. J. Tomatoes are now used in enormous quantities in the fresh state and head the list of all vegetables as a canned product. Thousands of bushels are also used in the manufacture of ketchups, chili sauce, and soups. The tomato is produced over a larger part of the United States than any other vegetable. It may be handled with few and simple appliances, and may therefore be canned in the home and in small factories where little capital is required, as well as in the large factories.

The development of a tomato suitable for canning purposes has been a specialty in itself. For this purpose the fruit should be moderately large, smooth, so that it will peel readily, ripened evenly to the stem, of a clear, red color, and having a large proportion of solid meat of good flavor. Varieties which ripen unevenly or are irregular in outline are difficult to peel and the percentage of waste is too high. Tomatoes which are yellow or purple do not have an attractive appearance on opening, and those with excessive seed cells or which are soft and watery will give the can the appearance of being slack filled or packed with water. A good pack is therefore dependent upon having a variety possessing the right qualities. The canner can not accept tomatoes of a half dozen or more varieties and get good results. He must therefore specify the variety grown or furnish the plants for his growers. The production of plants in hotbeds and cold frames to supply several hundred acres is of itself a very large task. The plants are grown in the field, the same as other crops, and a single large cannery will use the product of 1,000 acres. One ketchup manufacturer takes the entire product from more than 5,000 acres. A fair yield is 5 tons of fruit for an acre, but good cultivation and fertilization sometimes brings this up to 20 tons or more. Thirty-three bushels weigh about 1 ton.

At harvest time the fruit must be picked every day, or every other day, in order to insure collecting it when it is in its prime—just ripe, without green butts, and not overripe. It is preferable that the tomatoes be put in crates, which are wide and flat rather than deep, and which will hold not more than a bushel. They can be delivered to the factory in better condition in the flat crates than in the deep ones or in baskets, as the fruit will crush if piled in too many layers. The arrival in good condition lessens the time required for peeling as well as the loss in parts cut away. The tomatoes should be delivered to the factory promptly, as deterioration begins soon upon standing.

When the tomatoes are delivered at the factory they are weighed and inspection should be made of each load. One crate is taken out at random and dumped into a tank of water. All defective fruit can be detected at once, picked out, weighed separately, and the load docked accordingly. Rotten fruit can not be used and green fruit must be held to ripen. The separation at the factory entails extra expense in the inspection and sorting. The rotten fruit should not have been picked and the green should have been left in the field; the only way to reduce this waste to a mimimum is by means of a system of dockage.

The first step in manufacture should be proper sorting. This can be done better by a few persons than by the many peelers. Tomatoes which are green should be taken out and held in crates for one or two days, as may be necessary, but small green spots can be cut out by the peelers. The tomatoes with rot should be discarded. Tomatoes which are small, rough, misshapen, and sound, but which will not peel well, can be set aside for pulp. Such a separation will lessen the work and waste in the factory and in the end be economical. The sorting is best done upon a conveyer table, the tomatoes which are passed being fed directly into the washer.

The washing should be thorough and done without bruising or crushing the fruit. It is preferable that the fruit be dropped into a tank of water and rolled over and over gently, either by actually turning the tomato or by strongly agitating the water, and then spraying under a strong pressure as they emerge from the water. This latter operation is of greater importance than is generally supposed. As before stated, a comparatively large volume of water without force behind it is far less efficacious than a much smaller volume having force. The latter cuts the dirt and organisms off, the former only wets the skin and makes it look bright. Allowing tomatoes to dry in the sun after washing by each method will clearly demonstrate the difference. The water in the tank should be changed continuously by the addition of the water used in the spray, an overflow being provided for the tank. The majority of tomato washing machines are inefficient.

The tomatoes are scalded, while passing slowly through a tank or steam chamber, by the continuous action of hot water or steam. The scalding is only sufficient to loosen the skin and not to heat or soften the tomato. As the tomato emerges from the scalder it is sprayed with cold water, which causes the skin to split and arrests the heating of the fruit.

The clean-scalded tomatoes are delivered to the peelers in various ways, in pails and pans by carriers or belts, or by moving table tops, or they are delivered to the tables directly upon belts. Various devices have been used to carry the tomatoes to and from the peelers and to care for the waste, the object being to secure cleanliness and careful handling of the fruit. The bucket system is an old one and is in general use at small factories. The bucket is filled with scalded tomatoes and the peeler works from one bucket into another, dropping the refuse into a third bucket or into a trough under the table. The objection to the bucket is that the fruit on the bottom is mashed more or less before being reached by the peeler, and the same is true of the peeled fruit. Wide shallow pans have an advantage over the bucket in this respect. In peeling from the special tables, the tendency is to heap the bowls too full, which produces the same disadvantages found in using the bucket. Some paint the buckets different colors to indicate whether they are to be used for scalded tomatoes, peeled tomatoes, or refuse. All buckets or pans should be washed each time they are used, no matter how many times a day that may be. All tables and conveyers should be washed each time the plant stops, and oftener when needed.

The peelers hold the tomatoes with the stem toward the palm of the hand, pull the skin back from the blossom end, and close the operation by removing the core with the point of the knife, keeping it well directed toward the center so as not to open the seed cells. This is not only the quickest way to peel the tomato, but keeps it whole. Green and undesirable spots are cut out.

The cans are filled either by hand or by machine. The sanitary or open-top cans are filled by hand, as it gives a better appearance to the finished product. In this class the cans are weighed to insure the desired fill. If filled too full, which may easily happen, "springers" or "flippers" may result, and the product be unsalable though perfectly wholesome. "Springers" or "flippers," as before explained, have the appearance of a swell, but are not due to fermentation. Solder-topped cans seldom bulge in this way for the reason that they can not be sealed when too full, and, as a rule, they weigh from 3 to 4 ounces less than the hand-filled cans. Overfilling also necessitates a longer process, breaking up the fruit and detracting from the appearance of the product. In order to bring out the flavor some canners add one teaspoonful of a mixture of equal parts of salt and sugar, or of one part of salt to two parts of sugar, to each can. This is rarely done except upon high-grade goods and must be done by hand in order to insure uniformity.

There are several types of filling machines for solder-topped cans, which consist usually of a cylinder holding the quantity of tomatoes necessary to fill a can and a piston to force them in. The result is more or less badly broken fruit, though the contents are just as good as in the hand-packed. Some of the newer machines fill the cans on the principle of a collapsible tube, and the result is a decidedly better appearance. In all machine filling the measure is by volume rather than by weight. Cans which are filled full of whole tomatoes by hand are known as " hand-packed " or " solid-packed " in distinction from those filled by machine, or filled part full of whole tomatoes and juice added. The adding of juice is done for two purposes, one in high-grade stock to preserve the tomato whole or nearly whole, and in the standard grade to complete the machine fill or to utilize the entire product. In the first case the juice is taken from whole tomatoes and usually condensed slightly by boiling. In the latter case it is made from the trimmings and often of inferior quality. The use of water in canning tomatoes is unnecessary and is an adulteration.

Somewhat too much stress is being placed upon the quantity of solid meat which will be present after draining on a quarter-inch screen. A very high percentage of solid meat may mean the use of a variety which is hard and inferior, or fruit which is slightly green, in which event the flavor is deficient. The full, rich flavor of the tomato is not developed until it is thoroughly ripe, so ripe that the processing will cause a portion of the tissue to break down, and after long shipments they may be badly broken. While it is desirable to have a considerable proportion of the fruit whole or nearly whole, a broken condition is not of itself evidence of improper methods or poor quality. The cans are next run through an exhaust box, where they are subjected to steam heat for from 2 to 3 minutes, after which they are capped in the usual way. Tomatoes are given a process in boiling water for from 35 to 55 minutes.

Tomatoes are packed in No. 3 cans as a general rule, though they are also packed in all sizes from special cans for individual service on dining cars and cafés to the No. 10, or so-called gallon cans for hotel trade. Some of the latter are put up unpeeled. The No. 3 comes in the regular size and in what is known as extra tall. The tomato is also put up as condensed tomato, soup, paste, and purée. To produce these, the tomato is run through a "cyclone" to remove the hard portions and seeds, and then concentrated to different degrees. The use of condensed tomato or purée prepared from sound material has many advantages for some purposes over the regular canned article, and its use should be cultivated, especially for soups, etc. At the price paid for the standard grade of tomatoes a better article can be obtained as a purée or paste. Some purée is made from peel and waste from the canning. If the material is clean and sound there is no objection to its use, but too often this is not the case, as is made evident by the presence of microorganisms, broken tissue, and products of decomposition. A paste which is made from the whole tomato and from trimmings by a system of spontaneous fermentation and salting is used largely by foreigners. This article is no longer permissible in interstate trade. Another grade of paste is made by evaporating the pulp until it becomes very stiff and heavy. The straining of the juice or pulp from the seeds and hard portions can be done better and with less waste by special machinery than in the kitchen.

Tomatoes are sold under various trade grades, as extra choice, extra select, choice, select, extra standard, standard, and seconds. It is unfortunate that there are so many ways of designating the contents of a can, particularly when the prefix is meaningless. What one packer calls his "extra choice" or "extra select" may be no better than an extra standard or a standard of another packer. The real grade at present is dependent upon the packer's name, not upon what he claims. There should be but two grades—selected or first grade, and standard or field run for the second. A can of first grade tomatoes should be from selected, prime, ripe fruit, having a fleshy body, well-developed flavor, and uniform color. The can when opened should be full and most of the tomatoes whole or in large pieces, free from all peel, core, or defects. The net weight should not be less than 32 ounces in a No. 3 can.

A can of standard tomatoes should be from sound, ripe fruit, having a fair body and good flavor. The can when opened should be full, and part of the tomatoes whole or in large pieces. They should be well peeled and cored. The net contents of a No. 3 can should not weigh less than 32 ounces.

MARINE PRODUCTS.

There is a very large variety of fresh and salt water products put up in cans, and these have received the following classification by Charles H. Stevenson:[1]

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[1] The Preservation of Fishery Products for Food. United States Fish Commission Bulletin for 1898, p. 512.

There are five general classes of canned marine products, viz, (1) plain boiled, steamed, or otherwise cooked; (2) preserved in oil; (3) prepared with vinegar, sauces, spices, jellies, etc.; (4) cooked with vegetables, etc.; and (5) preserved by some other process, but placed in cans for convenience in marketing.

The first class includes salmon, mackerel, herring, menhaden, cod, halibut, smelt, oysters, clams, lobsters, crabs, shrimp, green turtle, etc.; sardines almost exclusively make up the second class.

The third class includes various forms of herring prepared as "brook trout," "ocean trout," etc., mackerel, eels, sturgeon, oysters, lobsters, crabs, etc.

The fourth class includes fish chowder, clam chowder, codfish balls, green turtle stew, terrapin stew, and devilled crabs.

The fifth class is made up of smoked herring, halibut, haddock, carp, pickerel, lake trout, salmon, eels, sturgeon, etc., and brine salted mackerel, cod, and caviar.

CRABS (CALLINECTES HASTA).

Canned crab meat in this country was the result of experiments made by James McMenamin, of Norfolk, Va. He began at Norfolk in 1878, but moved to Hampton in 1879, and that has been the chief point of supply up to the present time. The season for catching crabs is from April to October.

The live crabs are placed in large crates, well washed, and then run into a steam box, where they are cooked for 25 minutes. After cooling they are "stripped"—that is, the shell, viscera, and smaller claws are removed. The meat is then picked out of the bodies and large claws by hand, or it may be removed by centrifugal force or by compressed air. The latter methods, which are of recent origin, are effective and save much labor. In the centrifugal method the shell and claw are cut across to expose the cells and a quantity so prepared is placed in a centrifugal drum, almost the same as that used for drying in a laundry. The drum is made to spin at a high speed and all the meat is extracted. The compressed-air method consists of an air compressor and a storage tank, with pipes leading to a nozzle. The shell is held in front of the nozzle, the air is turned on, and the meat blown out. Either method is faster, better, and cleaner than the hand picking.

The meat is filled into cans and processed. The No. 1 cans generally used are first heated for a half hour in boiling water, vented, and then processed for 35 minutes at 240° F.

Crab meat is not so easy to keep as some other kinds, the tendency being to blacken more or less in the cans.

OYSTERS.

The oyster is a marine bivalve of the genus *Ostrea*, the species used in this country being *Ostrea virginiana*. It is found along the coast, chiefly in the shallow waters at the mouths of rivers and in bays. Chesapeake Bay has long been noted for the abundance of its oysters. They are found naturally all along the Atlantic coast as far north as

Massachusetts, and at one time were abundant in Long Island Sound. Active dredging depleted the beds and now the supply is maintained only by cultivation and restricting dredging operations. Some oysters are canned on the coast of Virginia, the Carolinas, and Georgia, but they are no longer canned north of Maryland. The oyster occurs in the Gulf on the west coast of Florida and along the shore to Texas. There is a large business in canning oysters in Mississippi and Louisiana. A few oysters are found on the Pacific coast, but not in sufficient quantity to warrant canning. The abundance of oysters in Chesapeake Bay made canning operations most profitable there, and the output acquired a reputation which still gives it some preference in the market. Prior to 1900 probably 95 per cent of the canned oysters were put up in Baltimore or in the immediate vicinity. The southern or Gulf oyster, however, has been proved to be equally good for canning purposes and the industry has rapidly assumed large proportions in those localities.

The oyster grows naturally on the hard reefs in from 15 to 180 feet of water, depending upon the temperature. In the Gulf they grow in shallower water. They will also grow in the bayous and flats by transplanting and furnishing shells or hard objects to which the spawn may become attached. Formerly no regulations were deemed necessary as to the places at which oysters might be taken, but since the rivers have become polluted with city sewage it is necessary to guard carefully against oysters from contaminated beds. The different States regulate the time when the fishing may be done, which is generally from the 1st of September until the 1st of May. The oysters for canning are usually taken from the beds between the 1st of October and the 1st of April.

Oysters were among the first products canned in this country. It is recorded that some were put up in an experimental way in New York in 1819, though they did not become a commercial proposition until the work was developed by Thomas Kensett in Baltimore in 1844. In the beginning all the oysters were shucked raw, by hand. In 1858 Louis McMurray, of Baltimore, found that by scalding the oysters in boiling water the shells would partially open and the labor of shucking could be lessened. Two years later the system of steaming them instead of scalding was developed, and no material change in method has taken place since that time. McMurray is said to have had a most excellent reputation as an oyster packer. His method was to save all the liquor and condensed steam from the steam boxes, filter it, and use it in filling the cans. He used neither salt nor water. There is probably no packer in the business at the present time following this method.

Oysters are obtained by dredging and by tonging, the former upon the reefs and in the deeper water, and the latter in the shallow bayous where planting has been done. The usual equipment consists of a schooner of about 48-foot keel, 55 feet over all, and 16-foot beam. When loaded, this will carry about 275 barrels of oysters. The crew consists of a captain and four men. A dredge is carried on each side of the boat and operated by two men. The dredge consists of a heavy iron rake about 3 feet wide, to which is attached a chain or heavy cord purse, the mouth of which is held open by an iron bar just above the rake. The dredge is lowered to the ground and dragged along by the movement of the boat. The rake loosens the oysters from the rock or ground and they are collected in the purse.

At short intervals the dredge is drawn on board by means of a windlass, the purse is emptied, and the operation repeated. The oysters are culled in some places, the small ones being returned. The catch is put in the hold if the boat is out in warm weather or is to be gone for more than a day. The trips are generally limited to from three to five days in order to insure delivery in a fresh condition at the cannery. Other varieties of smaller boats are also used, though power boats are generally barred. The Gulf-coast factories pay about 60 cents per barrel for oysters used in canning and 80 cents per barrel for those used in the fresh trade, owing to the difference in size. The barrel is rated by measure and not by weight. On the eastern coast the measurement is by the bushel.

The oysters are rated by size. If there are from 800 to 1,000 to a barrel they are known as standard, from 600 to 800 per barrel as selects, and from 450 to 600 per barrel as extra selects. The largest oysters, known as "counts" on the east coast or as "plants" on the Gulf coast, run less than 450 per barrel and are always sold raw. The larger oysters are found on certain reefs on which work has been prohibited for given periods or in certain water where planting has been done. The term "plants" when applied to eastern oysters refers to those taken from deep water, transplanted in shallow water, and cultivated until they have attained a desired size.

When the oysters are brought in, they are hoisted directly from the boat to the steaming car. These iron cars or crates are 28 inches wide, 19 inches deep, and 8 feet long. They will hold five barrels of 2½ bushels each. As soon as the car is filled the oysters should be given a thorough washing with clean water to remove the dirt and mud attached to the shell before it goes to the steam box, otherwise there is contamination during the shucking. The cars are wheeled from the dock to the steam box, which accommodates three cars. The steamer is a rectangular iron box, just large enough to admit the cars, and is 25 feet in length. There are a few variations from these sizes, but these are standard. The doors are closed at either end; steam is turned on until a pressure of 10 pounds is reached, and this is maintained for 5 minutes. The doors are then opened and the oysters allowed to cool quickly in the air. It is important that the oysters be steamed well so that there will be no shrinkage in the can, but not long enough to cause them to become crummy. Both the time and the temperature at which the steaming is done seems to have been fixed by experience, as none of the superintendents seemed to know what the effect would be if a lower temperature and longer time or higher temperature and shorter time were given.

The car of steamed oysters is pushed into the shucking shed, the shuckers standing around the car and working until it is emptied. The usual number of shuckers is from five to eight, and they are generally women and children.

The steamed oyster has the shell partly opened, the meat being easily removed by means of a short, heavy-bladed knife. The oysters are deposited in pans which are hooked to the oyster car. The shucker receives 5 cents for 3½ pounds of selects or 3 pounds of standards. The oysters are weighed as received from the shuckers, washed, and placed in cans by weight according to the grade and order. The cans are filled with a weak hot brine (2 pounds of salt to 10 gallons of water) by passing the cans through a dip box. This method was used at one time in other lines of canning, but has been superseded by more sanitary methods, and should be in this case.

The cans are capped in the usual manner, either by hand or machine, and are then processed in the retort at 240° F., the No. 1 cans for 12 minutes and the No. 2 for 15 minutes. The different packers vary the time a few minutes, but practically all use the same temperature.

The oysters are cooled as soon as sterilized, and when dry are ready to pack. The oyster is easily sterilized, it is not hard on the can, and there is little loss from spoilage.

The cans used are the No. 1 and No. 1 tall, or shanghai, and the No. 2 and No. 2 tall. These are given different fills, and here the consumer is the victim. The practice in regard to size and fill is best illustrated by the following price list obtained from packers:

Prices of the various grades and sizes of canned oysters.

Size and fill.	Price per dozen.	Price per ounce.
Standard oysters:		
No. 1 can, 1½ ounces	$0.37½	$0.25
No. 1 can, 3 ounces	.60	.20
No. 1 can, 4 ounces	.65	.16
No. 1 tall, 5 ounces	.70	.14
No. 2 tall, 6 ounces	1.30	.22
No. 2 tall, 8 ounces	1.40	.17½
No. 2 tall, 10 ounces	1.50	.15
Select oysters:		
No. 1 tall, 6 ounces	1.30	.22
No. 2 tall, 12 ounces	2.50	.21
Extra select:		
No. 1 tall, 6 ounces	1.50	.25
No. 2 tall, 12 ounces	2.90	.24

The variation in fill is not different from that of some other canned foods, but as there is nothing upon the label to inform the consumer concerning the contents it admits of unfair dealing. As the liquor is nothing but salt water and not juice, as many suppose, its use in excess of the amount necessary for proper packing of the article is not justifiable. It would undoubtedly be better for both packer and consumer if the cans were confined to the standard No. 1 and No. 2 and the "short" and "intermediate" weights eliminated. It is not possible to pack each can to weigh an exact amount, as some variation will take place in the water absorbed in processing, and a single oyster over or under weight, especially the large sizes, may cause a variation of a fourth of an ounce or more either way. They can be packed so that they will average the weights given, and the No. 1 should not weigh less than 4 ounces.

The term "cove" is applied to any canned oyster. It originally meant only the oysters obtained on the western shores of Chesapeake Bay and was distinctive of quality. Gradually any oyster became a cove oyster, and now it refers to canned oysters irrespective of where they are obtained.

SALMON.

Salmon canning on the Pacific coast is one of the large canning industries, and is of so much importance that Government aid is extended in maintaining fish hatcheries in order to keep up the supply. The first salmon canning was done on the Sacramento River in 1864, later on the Columbia River in 1866, in British Columbia in 1874, and in Alaska in 1882. The value of the salmon pack on the Pacific coast is more than $10,000,000 annually.

There are four species of salmon which have large commercial importance, *Oncorhynchus tschawytscha*, the chinook, quinnat, red spring, or King Alaska; *O. nerka*, the sockeye, blue back, or red fish; *O. kisutch*, cohoe, silver, or silver sides; and *O. gorbuscha*, humpbacks or pink Alaska. Preference is given to the bright pink color by the consumer, but for real quality the paler cohoe excels some of the others, the flesh being less dry and containing more oil and a better flavor.

The salmon are caught in the rivers as soon as practicable after they leave the sea on the way to the spawning grounds. They are caught by nets, seines, traps, and fish wheels. The catching of the fish is done on an elaborate scale, an idea of which may be gained from a brief description of a trap. This consists of a steel-wire netting, starting at the shore and carried out into the stream at an upward angle for a distance of about 2,500 feet. This netting is supported by piles placed about 15 feet apart. At the outer end is a large square compartment known as the pot. This is usually about 40 by 40 feet and in water as deep as 65 feet. This pot contains a dip net equal to its area. Just previous to reaching the pot the trap is made to zigzag or assume a heart shape, so that the fish in trying to pass up the stream will be directed into the pot. Adjoining the pot is a spiller, which is similar in construction, but of smaller size, having a tunnel or opening connecting the two. The fish pass from the pot to the spiller and are taken out by the dip net or brailer, which is 12 by 12 feet and is cast and drawn on board the boat by power, literally lifting out hundreds of fish at a time. They are hurried to the factory as rapidly as possible, where they are unloaded upon the dock by means of elevators or pews.

It is the general practice to permit the fish to remain out of water in bins for 24 hours before canning, as a certain amount of shrinkage takes place, otherwise there may be excessive blowing of the juice on venting. The fish are washed free from slime or gurry before they go to the butchering room.

The dressing of the fish, or butchering as it is called, is done speedily, mostly by machinery. The head and tail are sawed off on a band saw, where formerly they were cut off with a cleaver. The fish is then fed into the "chink" tail first and back down. By the revolution of this wheel, the fins are removed by special knives, the body is split open, the viscera torn out, and the interior wall scrubbed by revolving brushes. The dressed fish is delivered into a tank of water, and the offal delivered with the gurry. The iron chink does a better job than is done by hand, and is the most important machine in the canning of salmon. After the fish has been dropped into the tank of cold water, it is scrubbed thoroughly with brushes until it is clean.

The dressed fish is placed upon a special slitted elevator, which feeds it transversely into a series of revolving disks, which cut it into lengths corresponding to the height of the can. There are a variety of lengths used, but there are three which are standard; the No. 1 tall, No. 1 flat, and the half pound. Seven knives are used in the gang for cutting for tall cans, 13 knives for flat, and 17 knives for half-pound cans.

The grading of the fish is done on the basis of solid and less desirable body cuts. The filling of the choice parts is done by hand, and each can weighed. The short weights are supplemented by bits, but overweight is not reduced. Much of the filling, especially of the less expensive cuts, is done by machinery. The cans used must all be open top, and this is later either soldered or the joint made with a double seamer.

The solder capping of the cans is different from that practiced in other packing. First a piece of tin with the corners bent up is placed on the fish, then the can is set in a machine which wipes the upper edge, after which the end is put in place, and the can passed through another machine which crimps the end to the sides. This end contains a small hole or tip. The can then rolls, head downward, into a V-shaped groove which contains flux, and continues its rolling in another section of the groove containing solder, and it is here that the final sealing is done. The heating of the contents, due to the hot solder, causes some steam to be generated, and it is for the purpose of allowing this to escape that the piece of tin is placed within and under the vent. When the can leaves the soldering trough it is turned over and the vent closed or tipped. With sanitary cans no tin nor vent is needed, the cap being attached and sealed by machinery.

The cans are then placed in trays, the standard size being 35 inches square and 3 inches deep. Each tray will hold 160 tall or 86 flat standard No. 1 cans, the cans being on end in a single tier. The test for leaks is to set the tray in boiling water for a few seconds and watch for bubbles. Eight trays make a basket, and this constitutes a charge for the retort.

The process consists of heating at 220° F. for 30 minutes, then taking out the fish, venting, and retipping, and giving a subsequent heating for 1 hour and 15 minutes at 250° F. When open-top cans are used, the filled cans are run through an exhaust box very slowly so that they are thoroughly heated before the cover is attached. Venting becomes unnecessary, but the time of cooking remains unchanged—that is, the single heating is equal to both periods under the old method. The hot cans are immersed in lye to remove grease and oil and are then cooled in water. The net weight of the 1-pound tall or flat can should average 16 ounces.

SARDINES.

The sardines caught on the Pacific coast are much larger than those taken in the East and are handled in a different manner. They are caught in nets at night, and on being brought to the factory in the morning are put into bins and kept wet with running water for some hours. They are then dressed, scaled, heads and viscera removed, and again thoroughly washed in two or more changes of water. They are next dipped in strong salt brine for a few minutes, rinsed, and placed in wire trays to dry. In order to expedite the drying the trays are carried through a mechanical dryer so that all surface water will be removed. The crates are then dragged through a vat of boiling oil, the length of time being that necessary to cook the fish thoroughly, usually about 5 minutes. They are left in the crates until cool, which is usually until the following day, placed in the cans by hand, oil or sauce added to fill the interspaces, carefully exhausted, and processed at 240° F. for 1 hour and 15 minutes.

SHRIMP.

The shrimp is a crustacean and belongs in the same general class as crabs, crayfish, and lobsters. There are a number of varieties found in this country, but the one used for canning is the Gulf shrimp, *Panaeus brasiliensis.* The shrimp found in the fresh waters and west coast are used fresh, but are too small to be used in canning. The Gulf shrimp resembles a large crayfish and is from 5 to 7 inches long. They inhabit the deep waters and come to the shore twice each year. They are active swimmers and are provided with very long antennæ. The abdomen is the only part of the shrimp that is used, the head and thorax being thrown away.

The first attempt to can shrimp was made by Mr. G. W. Dunbar, of New Orleans, in 1867. His efforts did not meet with success until 1875, at which time he devised the bag lining for the cans. In 1880 a factory was started at Biloxi, Miss., and from that time to the present the majority of all the shrimp canned has been put up in these two cities. It is only within the past 10 years that the canning of shrimp has assumed considerable importance, but is still limited to about a dozen places in Louisiana and Mississippi. A cannery was started in Texas but failed to secure a regular supply, and the oyster canneries in Florida could not secure enough to make it profitable to prepare to receive them. The early supply of shrimp was obtained from Barataria bayou or lake, which gave the distinctive name, Barataria shrimp. The name is often improperly used now. The shrimp sent to England are called prawns.

Shrimp are caught in February, March, and April, and in September, October, and early November. The run is uncertain, and a catch depends upon the state of the weather; the quantity taken is very irregular. The shrimp are caught only in shallow water along the shore. Previous to this year (1911), all catches had to be made in less than 6 feet. Newer apparatus has been invented making it possible to take them in water 10 feet in depth. The shrimp are located by coursing over the ground in a small sailboat or a skiff and trying with a cast net. This is a circular net from 6 to 8 feet in diameter with leads every few inches around the edge and a cord attached for drawing it together. A man stands at the bow of the boat and makes trial throws until a school is located. When the shrimp are found the large seine is anchored on the shore at one end and the boat rowed out and around as large an area as the seine will cover. As soon as the second end is brought to the shore the men bring the two ends together and begin to draw in the seine. If the weights hang close upon the ground the chances for a catch are good, but if the seine should rise the shrimp will find a way out very quickly. The handling of the seine requires wading in water from

2 to 4½ feet in depth. The seine is drawn in such a manner as to cause the shrimp to go into the purse in their attempt to escape.

As soon as the catch is made safe the boat is brought alongside and the shrimp dipped out with scoop nets. They are stowed promptly in the hold of the vessel and well iced if the weather is warm or the trip is to continue for more than a day. The seines used in shrimp fishing are from 150 to 225 fathoms in length (900 to 1,350 feet) and from 140 to 150 meshes wide. (A mesh is three-quarters of an inch, giving a width of 105 to 112 inches.) The new apparatus for handling the seine consists of a stake with special pulleys near the bottom so that the seine may be drawn from below without a tendency to raise it off the ground.

The boat equipment for catching shrimp is essentially the same as for handling oysters, so that they are used interchangeably. The seine takes the place of the dredges and windlass, and the crew is usually made up of five or six men. The boats will carry about 140 barrels of iced shrimp.

Shrimps are weighed instead of measured, a barrel being 200 pounds. The pay for catching is $3.50 per barrel in the fall and $4 in the spring. The fall run is the more certain catch and requires less ice, which makes the difference in the schedule of prices.

When the shrimp are brought on the dock they are stored in ice until ready to use. The ice makes the peeling easier and is necessary to prevent spoilage. The removal of the head and shell is known as "peeling" the shrimp, and this is done for all canned shrimp. The head and thorax break from the heavy tail with ease and a slight squeeze will separate the fleshy portion from the shell. This work is done rapidly; the pay for peeling is about 1 cent per pound. The peeled shrimp are thoroughly washed in two or more changes of water and are then ready for blanching. The blanching consists in boiling the shrimp in salt water, which is done by suspending them in a wire basket in the boiling brine. The time of the blanch is usually about four minutes for the wet pack and five minutes for the dry pack. The salt in the brine is in the proportion of about 1 pound per gallon of water. Up to the time the shrimp go into the blanch they are white or slightly gray in color; the boiling in the brine causes them to become bright pink or red.

The shrimp are turned out upon trays having wire netting. As soon as cool they are filled into cans by hand, each can being weighed. The shrimp are all packed in either No. 1 or No. 1½ cans, the former having 4½ ounces and the latter 9 ounces. There is no attempt at grading.

Shrimp are put up in what are known as dry and wet packs. In the dry pack no liquor is added, while in the wet pack brine is used. The process for dry shrimp is 1 hour at 240° F. or 4 hours at 212° F.

for No. 1 cans, and 75 minutes at 240° F. and 4 hours at 212° F. for No. 1½ cans. The process for wet shrimp is 11 minutes for No. 1 and 12 minutes at 240° F. for No. 1½ cans.

The fill of 4½ and 9 ounces in the No. 1 and No. 1½ cans has the appearance of being light weight or slack filled. Experience has shown, however, that close filling causes matting of the shrimp and an unsightly appearance. The wet-packed shrimp are preferred by those who are familiar with the fresh article. They have better texture, odor, and taste than the dry packed. A barrel of good shrimp will pack 190 No. 1 cans or 100 cans of No. 1½.

Formerly shrimp were put up in bulk with a preservative. These were headless (only the head and thorax removed, the shell left on), and since that method of preservation is no longer approved, very few shrimp are obtained upon the market other than canned. Some pickled headless shrimp are put up in 1 to 5 gallon cans for hotels. These are boiled in strong brine for several minutes and put up in a saturated salt solution. They keep, but are very salty, and as it takes a long time to freshen them they are not available for immediate use.

Shrimp are difficult to keep. Put up in the ordinary tin can they will blacken in a short time and will attack the tin, making minute holes. Success in canning shrimp was dependent upon lining the can. This was first done by Mr. G. W. Dunbar, of New Orleans, in 1875. The method consisted in inserting a sack in the can and filling it with the shrimp to prevent their coming in direct contact with the tin. Later a thin veneering of wood, corn husks, parchment paper, asphaltum, and enamels were used. Parchment paper is used by all packers, with possibly one exception, at this time; in this case wood veneer is used.

MILK.

Canned, condensed, or evaporated milk is one of the large industries in this country. It is put up as plain or sweetened evaporated milk. The condensory is usually located in a good dairy section where a sufficient quantity of milk can be obtained by direct delivery in a very short time. The production must be under similar conditions to those obtaining in city dairying. The cows must be healthy, the dairy sanitary, the milking done in a cleanly manner, and the milk cooled and delivered promptly. The matter of cooling and prompt delivery is more important than in the city delivery, for the production of a slight acidity will interfere with condensing to a consistency where the product will comply with the law.

On being received at the condensory the milk is immediately tested for acidity and fat, and if the former exceeds 0.2 of 1 per cent the milk is rejected for regular trade, though it may be accepted at a lower price for making a cheaper grade for confectioners' use.

The milk is run through a clarifier to remove any foreign material not taken out by the home strainer. It is next drawn into a large tank which will hold an amount sufficient to charge the pan. The milk of several herds is mixed in order to secure uniformity in the fat content. Each tank is tested for fat and solids so that the exact ratio of concentration needed to give a certain result may be known. The milk is given a preliminary heating, usually to 190° F., though there may be some variation in the different plants. The milk is then ready for the vacuum pan, which consists of a large copper kettle completely hooded and connected at the top with a pump. The milk is heated in this kettle by means of a coil on the inside. The pump draws a vacuum of 25 to 29 inches and evaporation with violent agitation takes place at 130° F.; the temperature is usually kept below 150°. When the batch is nearly finished, a " strike " is made or a sample is drawn to test the consistency. The milk may or may not be run through a homogenizer to divide the fat into such fine particles that the cream will not rise in the finished product. It is next drawn into a filler, and in so doing the temperature may be raised to about 165° or 170° F., or the can may be filled cold.

The processing is done in retorts which differ from those used in vegetable packing in that special crates are provided which carry the cans and revolve so that the contents will be kept in a smoother condition. Both the time and temperature for condensation vary in different factories, and in careful work tests are made on a few cans before the batch is processed. In general it may be said that the temperature varies from 225° to 240° F., the time depending upon the degree of condensation desired, the condition of the milk, and the size of the can. Twenty minutes for a 6-ounce and 28 minutes for a 12-ounce can at 240° F. is safe. Immediately at the close of the cooking the cans are cooled, placed in cases, and shaken for three minutes.

Condensed milk can not be used for all the purposes of fresh milk, and is somewhat less digestible than the fresh on account of the cooking. It is an excellent substitute for many purposes and the evaporated—that is, the unsweetened product—has the advantage of being sterile.

SPECIALTIES AND SOUPS.

BEANS, BAKED.

Pork and beans, beans and tomato sauce, and baked beans are the ways which the labels read on the product which a few years ago was known only as " baked beans." The beans used for this purpose are the small white pea or navy bean. They are chiefly grown in New York, Michigan, and Wisconsin and are a regular field crop, sowed, cultivated, and harvested when ripe and used only in the fully ripe dried state. The quantity used in this way is enormous.

The beans should be of good quality, small, white, machine-cleaned, and hand-picked for defects. The first step in preparation is soaking, and this is done in tanks or barrels and lasts for from 12 to 24 hours, depending upon the method of handling. The water is changed in the tank about once in 6 hours, or, on the fancy article, about once in 4 hours.

From this point on the preparation varies greatly in different factories. For the very cheap trade the beans are boiled in a squirrel cage or pea blancher for a few minutes before placing them in the can; others boil them very slowly in an iron-jacketed kettle from 30 minutes to 3 hours before canning. Some boil them just long enough to slip the skin, the length of time depending wholly upon the grade of the bean.

Before the cans are filled, a piece of pork is placed in the can, then the beans, and finally the sauce. The sauce varies greatly, though tomato sauce is the most popular at present. This is made from a good heavy pulp, salt, sugar, and spices, the proportions being varied to suit the fancy of the packer. Plain sauce is made with water, salt, sugar, molasses, and spice. It is important that just the proper quantity of sauce be added, for in the processing some moisture will be taken up by the beans, and if too little sauce or moisture is added they will be dry and hard, while if an excess be added they will be sloppy.

In these methods there is no real baking, the beans having been soaked and boiled. They are subsequently heated in the can at a baking temperature, but no moisture can escape, and baking generally implies that the material is subjected to dry heat, usually in an oven. The real characteristic is the change in and breaking up of the tissues with loss of weight, due to the escape of moisture. Formerly baking was done under hot ashes or coals, in clay or brick ovens; now it is done in stoves and special ovens, and the latter may be heated by steam. The same results may be accomplished in superheated steam as in hot air. The difference between baking and roasting is not always clear, but between baking and boiling there is a distinction. The term "baked" beans, therefore, implies that they have been exposed to a dry heat. This is accomplished by heating the soaked beans for a short time, until they soften but do not break open or become mushy. They are then placed in large pans in thin layers and allowed to bake in ovens until they become dry and mealy and develop the characteristic brown color. The beans, when poured upon the filling table will readily separate from one another. Another method is to place the beans in large trays in the retort and subject them to dry steam until dry and mealy. The result is almost the same as in the oven—a loss of about 8 per cent in weight taking place and giving the same dry baked bean. These are filled in the can and sauced, as has already been described.

The processing of beans will depend altogether upon the method of preparation, usually from 1 hour to 2½ hours for a No. 2 can, at a temperature of from 245° to 250° F.

There is probably no staple canned which presents more variety in quality and flavor than the bean. The best is a high-grade product, the beans used are expensive, and the dressing, if made of tomato, is good pulp, the same care being given in its preparation as is used in preparing any other. Not so much can be said for some of the very cheap brands, the beans used are inferior, the pulp used is from trimming stock, and the object is to get as much water in the can as possible. The net weight of beans in a No. 1 can should be not less than 19 ounces.

HOMINY.

Canned hominy is used in every mining and logging camp in the country. It is primarily the diet for the hard worker, but is also used with milk to take the place of a breakfast food in thousands of homes. It was first packed in 1895 by Mr. I. V. Smith, of Delphi, Ind., and almost immediately others followed.

Hominy is made from selected white corn. The shelled grain is screened to take out all small, defective, or split grains, and any chaff or foreign substance. It is then washed and run into the lyeing machine. Here the corn is treated with a hot solution of lye, during which time it is constantly cooked and agitated until the tough hull loosens. The strength of the lye and the length of time required for the cooking varies at different factories; the time of cooking varies from 20 to 45 minutes. After the lye has accomplished its work the grain is run through a huller, which is in reality a short conical "cyclone," which removes the hull and tips.

The grain is next washed in a squirrel cage, pea blancher, or hominy washer. The different canners use very different methods at this point. Some soak the corn over night in order to have the kernels swell to the maximum before canning; others soak and cook for only a short time, an hour or two; while some fill the cans at once and depend upon the swelling in the process to give the desired result. The soaking has the effect of getting rid of traces of lye, makes a more tender kernel, and a clearer liquor. The cans are so filled that when the process is completed the grains fill the can nearly full and should be covered by only one-fourth inch of liquor. The liquor should be fairly clear and few black tips present.

The standards adopted for hominy by the Indiana Canning Association are—

No. 3 Fancy: Minimum weight after draining a can, 22 ounces, and to fill to one-half inch of the top of the can. Not more than 2 per cent of black tips. Prepared from selected white corn.

No. 3 Standard: Minimum gross weight, 39 ounces, 18 ounces of hominy after draining, and can to be filled to 1 inch of the top when drained. Prepared from medium-sized white corn and to contain not more than 5 per cent of black tips. This weight is too low and should be not less than 20 ounces in a No. 3 can.

SAUERKRAUT.

Sauerkraut is made by the natural fermentation of cabbage in casks. The cabbage heads are stripped of all outside or green leaves, leaving only the white sound head. It is then cut into thin slices in a specially constructed machine. The long, fine-cut cabbage is evenly spread and well packed in casks. To each layer salt is added at the rate of about 2 pounds per 100 pounds of cabbage. The salt is used as flavoring and to modify in some degree the fermentation. If too much salt is used, a pinkish color results; if too little, the fermented product may become more or less slimy. The temperature of the weather at the time of putting up the cabbage also influences the fermentation. If the weather is very warm, the fermentation is too rapid, the product has a very white but more or less slimy appearance, and the cabbage is tough rather than of a natural crispness. If the temperature is very low, fermentation will be arrested. The best temperature is probably between 60° and 70° F., and the process requires about 4 weeks. Fermentation begins as soon as the cabbage is placed in the cask, but there is only a slight rise of temperature as compared with most fermentation processes. A heavy foam rises to the top, which must be skimmed off every day, and when this ceases to form the brine goes down and the process is complete. Use can be made of the kraut at once, though it seems to be better after standing. The kraut will keep in the casks for a long time, provided there is no leakage, and the spoilage is usually limited to a few inches on the top.

Kraut is easily canned, which is the only clean way of dispensing it in groceries in small quantities. The canning should be done where the kraut is made. The shipping of kraut in barrels to distant points to be canned has nothing to commend it and much to condemn it. The repacking in barrels means labor and loss of material, and in too many cases the loss of natural brine, after which spoilage takes place easily. The canning should be done while it is in the freshest possible state at the point of production. Kraut is easily kept. The cans should be filled full, weighed, and sufficient hot water added to fill the can; then exhausted, capped, and processed at boiling temperature for 25 minutes.

A properly filled No. 3 can should not contain less than 22 ounces of kraut, as determined by emptying upon a sieve of one-eighth inch mesh and allowing to drain for two minutes.

SOUPS.

Soups of almost every description may be obtained in cans. There is no standard, but each one is made according to the formula of the particular packer. Some soups are concentrated, while others are ready for use. They are practically all packed under Government inspection, both of the plant and the materials used. No meat products can enter interstate trade without being inspected, and since nearly all soups contain either meat or stock made from meat, they must comply with all the requirements governing meat inspection.

Soups are classed as meat or vegetable, though there are but few of the latter that are not made from some kind of meat stock. The usual procedure in making soup is to select the meat stock, which is usually beef, though veal or mutton may be added. The meat used by some of the best factories is of the very highest quality, not merely any meat which has passed inspection. This is cut into pieces, the size depending upon whether it is to be used in the soup or only for the stock, and is placed in large steel kettles. These are heated by steam and covered tightly, so that the stock may be cooked slowly without evaporating. The cooking is continued below the boiling point for several hours, depending upon the kind of meat used and the care given to the making of the soup. The slow cooking has the effect of bringing out the extractives, giving a better flavor and a richer product. The liquor is skimmed at regular intervals, and if the stock is for a clear soup or a bouillon, it is clarified with eggs and filtered. If for a soup containing the meat, this last operation may be omitted.

The vegetables used in making soups are carrots, turnips, parsnips, peas, beans, onions, leeks, celery, okra, tomatoes, etc. As far as possible, these should be used in their fresh state, but as it is not possible to have them all fresh at the same time the canned article must be substituted. The vegetables used are prepared separately, washed, peeled, cut into pieces, cubes or special forms, blanched, and in some cases given a separate cooking to get the proper tenderness. These are mixed in the proportions desired, placed in the cans by weight, and the stock added afterwards. The process will depend upon the body, whether thick or thin, and the quantity of meat used.

The making of soups is peculiarly a chef's work; it is not possible to give a formula for so many pounds of meat and vegetables, set a definite time for cooking each, and get a first-class product. The characteristic flavoring depends upon the blending and the condiments used, which is a matter of training and judgment. For meat soups the best packers follow the practice of holding the cans in stock for some weeks in order that they may improve on standing. A good soup requires much work in its proper preparation, much more than is given in the canning of fruits or vegetables. Many soups are made according to formula, and while of good material, are not distinctive.

A list of soups includes the following: Beef, bouillon, celery, ox-tail, mock turtle, veal, chicken, chicken gumbo, consommé, green turtle, clam broth, clam chowder, mutton broth, tomato, tomato-okra, vegetable, pea, asparagus, mulligatawny, vermicelli, and Julienne.

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U. S. DEPARTMENT OF AGRICULTURE.

BUREAU OF CHEMISTRY—BULLETIN No. 152.

R. E. DOOLITTLE, Acting Chief of Bureau.

PROCEEDINGS

OF THE

TWENTY-EIGHTH ANNUAL CONVENTION

OF THE

ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS,

HELD AT

WASHINGTON, D. C., NOVEMBER 20-22, 1911.

EDITED BY

HARVEY W. WILEY,
SECRETARY OF THE ASSOCIATION,

WITH THE COLLABORATION OF

A. L. PIERCE,
Editor, Bureau of Chemistry.

WASHINGTON:
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LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Chemistry,
Washington, D. C., February 5, 1912.

Sir: I have the honor to submit for your approval the Proceedings of the Twenty-eighth Annual Convention of the Association of Official Agricultural Chemists. Owing to the steadily increasing volume of the work covered by these investigations and the importance of the detailed studies of the methods of analysis, which now are generally recognized as official in inspection work, only the reports and correlated papers, together with the specific action on the part of the association affecting the conduct of the work, are presented, all discussion being omitted. I recommend that this report be published as Bulletin No. 152 of the Bureau of Chemistry.

Respectfully,

H. W. Wiley,
Chief of Bureau.

Hon. James Wilson,
Secretary of Agriculture.

CONTENTS.

Monday—Morning Session.

Page.
Members and visitors present 5
Report on phosphoric acid. By H. D. Haskins and A. J. Patten 10
Neutral ammonium-citrate solution. By A. J. Patten and C. S. Robinson 25
Report on nitrogen. By James W. Kellogg 27
Report on potash. By E. L. Baker 28
Estimation of potassium as the cobalti-nitrite. By W. A. Drushel 42
Some features of the cobalti-nitrite method for potassium. By Leon T. Bowser. 45
Report on soils. By J. G. Lipman and G. S. Fraps 50
Determination of carbon dioxid in soils. By Leon T. Bowser 56
Note on pot experiment showing gains in nitrogen when certain legumes were grown. By B. L. Hartwell and F. R. Pember 59
Report on inorganic plant constituents. By O. M. Shedd 60

Monday—Afternoon Session.

Report on insecticides. By C. C. McDonnell 68
Report on water. By W. W. Skinner 72
Report of Committee A on recommendations of referees. By J. P. Street 83
Report of committee on amendments to the constitution and by-laws. By L. L. Van Slyke 86

Tuesday—Morning Session.

Report on food adulteration. By A. S. Mitchell 88
Report on spices. By R. W. Hilts 89
Index of refraction of the ether extract of paprika and pimenton. By D. L. Weatherhead 95
Report on fats and oils. By H. S. Bailey 96
Report on the adulteration of dairy products. By A. E. Paul 100
Report on cereal products. By H. L. White 101
The effects of time and temperature of digestion on the amounts of acidity and nitrous nitrogen extracted from flour. By A. L. Winton and A. W. Hansen . . 114
A recalculation of Juckenack's egg-noodle tables. By R. W. Hilts 116
Note on determination of tin in foods. By A. W. Hansen and L. C. Johnson . . 117
Report on condiments other than spices. By W. J. McGee 118
Report on colors. By W. E. Mathewson 122
Report on vinegar. By W. A. Bender 125
Report on flavoring extracts. By R. S. Hiltner 127
The chemical composition of authentic vanilla extracts, together with analytical methods and limits of constants. By A. L. Winton and E. H. Berry 146
Report on cocoa and cocoa products. By W. L. Dubois 159
Report on tea and coffee. By M. E. Jaffa 163
Report on preservatives. By H. E. Barnard 167
Report on water in foods. By H. C. Lythgoe 167
Methods of analysis of fruit products. By A. E. Paul 168
Report of committee on cooperation with other agricultural organizations. By H. W. Wiley 170
President's address. By F. W. Woll 172

TUESDAY—AFTERNOON SESSION.

Page.
Report on the separation of nitrogenous bodies (meat proteids). By C. R. Moulton........ 176
Notes on the determination and valuation of potash. By P. F. Trowbridge.. 184
Report on the separation of nitrogenous bodies (milk and cheese). By A. W. Bosworth and O. B. Winter........ 185
Report of Committee C on recommendations of referees. By A. L. Winton... 188
A note on the determination of benzaldehyde in liqueurs, distilled liquors, and cordials. By F. G. Smith........ 192
Note on a tentative method for the determination of essential oil in alcoholic solutions. By R. S. Hiltner........ 195

WEDNESDAY—MORNING SESSION.

Report on feeds and feeding stuffs. By G. M. MacNider........ 197
Report on sugar and molasses. By William E. Cross........ 202
Temperature corrections in raw sugar polarizations. By W. D. Horne........ 207
Report of Committee B on recommendations of referees. By E. M. Chace.... 210
Report of the auditing committee. By J. M. Bartlett........ 212
Report of the committee on resolutions. By Wm. Frear........ 213
Report on meat and fish. By Ralph Hoagland........ 213
Report on fruit and fruit products. By A. W. Blair........ 218
Report of committee on nominations. By W. A. Withers........ 220
Report on tannin. By J. S. Rogers........ 221
Report on medicinal plants and drugs. By L. F. Kebler........ 234
Report on headache mixtures. By W. O. Emery........ 236
Report on medicated soft drinks. By H. C. Fuller........ 241
Estimating small quantities of morphin in mixtures. By E. O. Eaton........ 242
Standard for tincture of ginger. By L. F. Kebler and C. H. Kimberly...... 244
Note on assay of nitroglycerin tablets. By A. G. Murray........ 248
Officers and referees of the Association of Official Agricultural Chemists, 1911–12. 251
Constitution........ 253
By-laws........ 255
Index........ 256

ILLUSTRATIONS.

Page.
FIG. 1. Agitating apparatus for phosphoric acid determination........ 14
2. Apparatus for determination of carbon dioxid in soils........ 57
3. Spray trap........ 239
4. Alcohol distillation apparatus........ 240

PROCEEDINGS OF TWENTY-EIGHTH ANNUAL CONVENTION OF ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS.

FIRST DAY.

MONDAY—MORNING SESSION.

The twenty-eighth annual convention of the Association of Official Agricultural Chemists was called to order by the president, Mr. F. W. Woll, of Madison, Wis., on the morning of November 20, at the Raleigh Hotel, Washington, D. C. The following members and visitors, 225 in number, registered:

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REPORT ON PHOSPHORIC ACID.

By H. D. Haskins, *Referee*, and A. J. Patten, *Associate Referee*.

Review of Work Done on Basic Slag.

The work of the referee has consisted of a study of the Wagner method and its various modifications to determine if the method was applicable to the analysis of basic slags, particularly with reference to the determination of available phosphoric acid. This work seemed to be in line with the recommendations of committee A for the year 1909, "That the Wagner method for the estimation of the availability of phosphoric acid in slags be brought up for provisional adoption at the next meeting." No report on phosphoric acid was made for the season 1910 and action by the association as to the adoption of this method was deferred another year.

As considerable work on basic slag has been done from time to time by members of the association, it may not be out of place here to give a brief abstract of such work extending over a period of 15 years. In the proceedings for 1896 H. A. Huston presented a paper on the action of ammonium citrate and citric acid on basic slag. It consisted of a study of the influence of time on digestion, influence of temperature, influence of acid and alkali, influence of quantity of slag used. A comparison was also made of the Wagner method (acidulated citrate of ammonia) with the official method. A paper was also presented by H. W. Wiley on the mechanical analysis of basic slag.

In 1897 a comparison was made by H. B. McDonnell of the availability of phosphoric acid in basic slag by Wagner's method, ammonium citrate, and a 1 per cent citric acid solution. In the Proceedings for that year, upon recommendation of the committee, a description of the Wagner method was published (see pp. 72 and 73).

In 1898 H. J. Wheeler reported that at a meeting of representatives of German experiment stations which he had attended it was proposed by Wagner that a 2 per cent citric acid solution be adopted in place of the acid citrate solution previously employed, the conditions of treatment remaining the same. Wagner's suggestion was strongly supported by Maercker, of Halle, and others. The reason for the proposed change was the fact that it was difficult to secure comparative results, even on the same samples, in the various German laboratories.

At the association meeting in 1900 Mr. Macfarlane, of Canada, brought out the fact that much slag was being sold in both Canada and the United States, and emphasized the fact that the association had no adequate method of determining the available phosphoric acid in this material. He gave some analytical data and urged the adoption of some method. This was favored by Myers and some others but met with opposition. It was at this meeting that the suggestion was made concerning the commercial valuation of slags by mechanical analysis and total phosphoric acid.

In 1901 H. K. Miller included a sample of basic slag in the samples sent out for cooperative work, with instructions to analyze by a 1 per cent citric acid solution. No recommendations were made, however, concerning the analysis of slags.

In 1902 C. H. Jones as referee studied slags by the official method, the 1 per cent citric acid solution, 2 per cent citric acid solution, ammonium citrate solution diluted with an equal volume of water, and the Macfarlane method. The latter provided for the removal of the free lime in the slag by boiling with a

5 per cent ammonium chlorid solution and treating the residue with 100 cc of a 1 per cent citric acid solution, shaking every five minutes for one-half hour and determining the phosphoric acid in the washed residue. No recommendations were made, but it was suggested that work on basic slags be discontinued on account of the limited use of slags in this country.

In 1903 Hilgard took exception to the statement made in the Proceedings of the previous year "that very limited quantities of slag were used in this country" and stated that over 1,500 tons of it were used in California alone the previous year. He urged that, as the slag was guaranteed by the Wagner method, some suitable method should be provided for its analysis. A resolution was therefore adopted that the referee on phosphoric acid be instructed to reconsider the question of the valuation of basic slag, especially the recommendation to establish a standard based on the total phosphoric acid and degree of fineness.

No work was done in 1904, but the following year Mr. Williams as referee planned work on three samples of slag by four separate methods: The ammonium chlorid citric acid method, the cane sugar citric acid method, the citric acid method, and the neutral citrate of ammonia method. The recommendations were that the referee next year plan field and pot experiments with cultivated plants to study the relative value of phosphoric acid in foreign and domestic slags, redonda, and precipitated phosphates; also that further study be made on determining the total and available phosphoric acid in basic slags.

In 1906 no work was done on slags. George D. Leavens urged the adoption of some suitable method of determining available phosphoric acid in slags. It was recommended by committee A that the next referee of phosphoric acid take up methods applicable under American conditions for the examination of basic slags.

In 1907 Kilgore recommended that the fineness of the material be determined according to the plan followed with bone meal, and the commercial value estimated on the basis of total phosphoric acid and fineness of product. This recommendation, which was approved by committee A, was adopted provisionally, pending further work. The following recommendation was also made by committee A and was adopted, namely, that the referee further study the subject of basic slag with a view to devising some method for the determination of the available phosphoric acid which it contains.

In 1908 H. D. Haskins, in a paper on the mechanical analysis of slags, showed the importance of specifying the sieve mesh to be used in mechanical analysis of slags and also showed the inconsistency of the mechanical method of valuing, provided the slag was adulterated with floats or other finely ground mineral phosphate.

In 1909 no work was done, but committee A recommended:

First. That a special committee be appointed to confer with the various experiment stations to secure cooperation in cultural experiments to determine the available phosphoric acid in basic slag; also to make an annual report to the association and at the end of five years to make appropriate recommendations concerning laboratory methods.

Second. That the Wagner method be brought up for provisional adoption at the next meeting.

No work was done and no recommendations were made at our last meeting.

Acting upon the recommendation contained in the last referee's report on phosphoric acid, which was sustained by committee A, the plan of your referee has been to make a systematic study of the Wagner method with its various modifications and in this work to enlist the cooperation of as many analysts as possible. In response to the circular letter sent out by the secretary, 10 different chemists volunteered to cooperate in the work. Eleven other men promised cooperation, in response to personal letters sent out by the referee.

Two samples were prepared and sent with the instructions during the month of March. No. 1 was a basic slag phosphate imported from Germany. No. 2 was composed of equal weights of basic slag phosphate and ground Florida rock phosphate, the two being intimately mixed. The instructions for the work were as follows:

INSTRUCTIONS FOR COOPERATIVE WORK OF 1911 ON AVAILABLE PHOSPHORIC ACID IN BASIC SLAG PHOSPHATE.

DEAR SIR: There are being sent to you by express two samples marked 1 and 2. Please have them analyzed as per methods outlined below.

1. Determine moisture at 100° C.

2. Determine total phosphoric acid on each, according to official method, using the *a_7* method of making solution. (See Bul. No. 107, Revised, Bureau of Chemistry.)

3. Determine total phosphoric acid by adding to 50 cc of the filtrate 50 cc of citrate of ammonia mixture. (See solution 5.) If the solution is still acid, make neutral with ammonium hydroxid and add 25 cc of magnesia mixture. (See solution 4.) Stir 30 minutes, allow to stand two hours, filter through a platinum Gooch crucible, wash six times with 2 per cent ammonium hydroxid, and proceed as usual.

4. Determine available phosphoric acid by 2 per cent citric acid (Wagner's method), as follows:

(A) *Making the citric solution.*—Weigh 5 grams of the basic slag, transfer to a one-half liter flask containing 5 cc of 95 per cent alcohol, and make up to the mark with dilute citric acid solution (2 per cent) of a temperature of 17.5° C. Fit the flask with a rubber stopper, and put at once into the rotary apparatus for 30 minutes, making 30 to 40 revolutions per minute. Take off and filter immediately.

(B) *Analysis of the citric solution.*—As soon as the filtration is completed, analyze the solution at once according to the three following methods:

(*a*) *Molybdate method.*—Take 50 cc of the clear filtrate and add to it 100 cc of molybdate solution. (See solution 3.) Put the beaker into a water bath until the temperature reaches 65° C.; take out and allow to cool at ordinary temperature. Then filter, and wash the yellow precipitate of phosphomolybdate of ammonia with 1 per cent nitric acid. Now dissolve in 100 cc of 2 per cent ammonium hydroxid (cold) and add to the solution 15 cc of magnesia mixture (see solution 4), drop by drop during continuous stirring; then cover the beaker with a glass cover and allow to stand for about 2 hours. Then filter the double phosphate of ammonia and magnesia through a tared platinum Gooch crucible, wash 6 times with 2 per cent ammonium hydrate, dry, and proceed as customary for phosphoric acid determination.

(*b*) *Citrate of ammonia-magnesia mixture method.*—Put 100 cc of the clear filtrate into a 200 cc flask and add 50 cc of citrate magnesia mixture. (See solution 6.) Heat the flask slightly (about 15 minutes) by means of a Bunsen burner until the silicic acid has been precipitated. Shake the flask in order to conglomerate the precipitate, and continue heating to boiling point. Allow to cool, add 25 cc of hydrochloric acid of 1.124 specific gravity, and allow it to stand for about thirty minutes, during which time shake occasionally. Fill up to the mark with water, fit the flask with a rubber stopper, and shake vigorously several times till the precipitate of silicic acid has been divided into very fine particles. Then filter and add to 100 cc of the filtrate (0.5 gram basic slag) 50 cc of ammonium hydroxid (10 per cent) while stirring the contents of the beaker. Continue stirring, preferably by means of a stirring apparatus, for thirty minutes, filter the precipitate, and treat as usual.

(*c*) *Iron citrate of ammonia-magnesia mixture method.*—To 50 cc of the clear filtrate add at once 50 cc of iron citrate of ammonia-magnesia mixture. (See solution 7.) Cool rapidly to room temperature, stir for thirty minutes in a stirring apparatus, allow to stand for two hours, filter through tared platinum Gooch crucible, wash six times with 2 per cent ammonium hydroxid, and treat as usual.

PREPARATION OF SOLUTIONS.

1. *Concentrated solution of citric acid (10 per cent).*—Dissolve in water exactly 200 grams of chemically pure crystallized citric acid having its full percentage of water of crystallization. Make up this solution exactly to 2 liters. (Where a large number of analyses are to be made, 0.5 gram of salicylic acid should be added to the liter of this solution so as to preserve it.)

2. *Dilute solution of citric acid (2 per cent).*—Mix exactly 1 volume of concentrated solution of citric acid (solution No. 1) with four volumes of water. The resulting solution should have a temperature of about 17.5° C. when used.

3. *Molybdate solution.*—Put 125 grams of molybdic acid into a 1-liter flask with about 100 cc of water and dissolve by adding, while shaking, about 300 cc of 8 per cent ammonium hydroxid. Mix this solution with 400 grams of nitrate of ammonia, fill up to the mark with water, and add the whole to 1 liter of nitric acid of 1.19 specific gravity. Allow to stand for 24 hours at a temperature of 35° C. and then filter.

4. *Magnesia mixture.*—Dissolve 110 grams of pure crystallized muriate of magnesia and 140 grams of muriate of ammonia in 1,300 cc of water, and mix this solution with 700 cc of liquor of ammonia containing 8 per cent of ammonium hydroxid. Allow this mixture to stand for several days and then filter.

5. *Citrate of ammonia.*—Dissolve 100 grams citric acid in 350 cc of 24 per cent ammonium hydroxid and make to volume of 1 liter.

6. *Citrate of ammonia-magnesia mixture.*—Place 200 grams of citric acid and 40 grams of muriate of ammonia in a 1-liter flask, and add 200 cc of water, and then 500 cc of liquor of ammonia (20 per cent). Keep the flask stoppered until the contents are dissolved and cooled down. Then add 55 grams of muriate of magnesia, and fill up to the mark with water.

7. *Iron citrate of ammonia-magnesia mixture.*—To 1,000 cc of citrate of ammonia-magnesia mixture add 10 cc of the 20 per cent ferrous chlorid solution.

8. *Ferrous chlorid solution.*—Dissolve 20 grams of ferrous chlorid in 100 cc of distilled water.

FURTHER INFORMATION AND PRECAUTIONS TO BE TAKEN.

1. A photograph and detailed drawings of an inexpensive but efficient shaking apparatus accompanies the instructions for phosphoric acid work (see fig. 1). This apparatus has been in use for a number of years in the laboratory of the Massachusetts agricultural experiment station; it can be easily made by your local carpenter, and should not cost over $8 or $9; it also answers every purpose and obviates the necessity of purchasing an expensive apparatus listed in various catalogues at from $27 to $35.

2. It will sometimes be seen, when pouring the 500 cc of the citric-acid solution onto the 5 grams of basic slag, that the latter clogs somewhat, and becomes permeated only after a considerable time. In order to guard against this, 5 cc of alcohol are poured into the half-liter flask before the basic slag is put in; this insures immediate and complete permeation.

3. The rotary apparatus prescribed for shaking the flasks must not be substituted by ordinary shaking or rocker apparatus, as the latter differs in construction and effect.

4. The half-liter flasks (after the design of Wagner) must have a neck width of at least 20 mm and are marked at least 8 cm below the mouth. These two points are important; for if the neck width is too narrow and the mark too high, the result will be too low, owing to the movement of the liquid being so limited.

5. The rotary apparatus must turn round its axle 30 to 40 times per minute. Variation within these limits has no marked influence on the results.

6. The filtration must be done immediately after 30 minutes' rotation, and it is recommended to use a folded filter paper of such size that the whole quantity of liquid can be poured onto the filter at once. Small and bad filtering papers give rise to error, in consequence of too slow filtration. If at first the

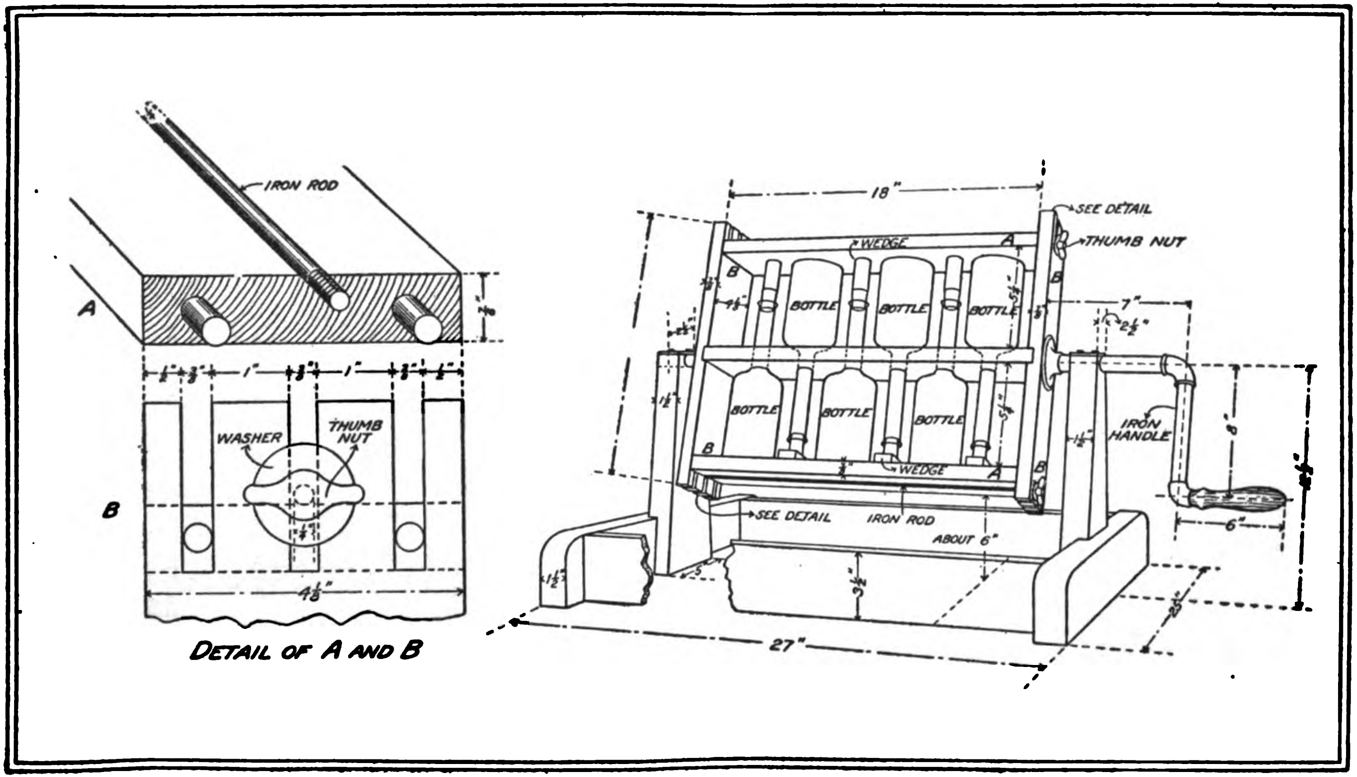

Fig. 1.—Agitating apparatus for phosphoric acid determination.

filtrate is not clear, it must be again filtered (through the same filter) until it becomes clear.

7. After filtering the citric solution of the basic slag, the precipitation of the phosphoric acid should be carried along without delay, as long standing increases the tendency of the silica to precipitate during the operation.

8. If the beaker containing the mixture of phosphatic and molybdic solutions is put into the water bath until the temperature reaches between 60° and 70° C., a precipitate free from silicic acid results. If heating is continued for a considerably longer time, the precipitate will often be mixed with silicic acid, especially when the molybdic solution is not added to the filtrate immediately, but only after 6 to 12 hours (or longer) after filtration. If silicic acid is present, the precipitate dissolves slowly in ammonium hydroxid, but at first not clearly. Special attention must be paid to the point that the yellow precipitate is dissolved quickly and quite clearly by ammonium hydroxid (2 per cent) not made warm. If the solution becomes clear only after some time, molybdic solution and nitric acid must be added to same in order to get a pure precipitate of phospho-molybdate of ammonia; in other words, the phosphoric acid must be reprecipitated by the molybdic solution.

Please return results to the referee as early as possible. The samples have been sent out early so that the work may be taken up before the rush of the fertilizer inspection begins in the various States. It is to be hoped that results may reach the referee by the first of August at the latest. An earlier report than this will be appreciated.

Very truly yours,

H. D. Haskins,
Referee on Phosphoric Acid.

TABULATED RESULTS OF COLLABORATIVE WORK.

TABLE 1.—*Comparative work on basic slag.*

COMPARATIVE RESULTS.

Chemist.	Moisture.		Total phosphoric acid.				Available phosphoric acid.					
			Sample 1.		Sample 2.		Sample 1.			Sample 2.		
	No. 1.	No. 2.	Official method.	Special method.	Official method.	Special method.	Molybdic method (a).	Citrate of ammonia-magnesia mixture method (b).	Iron citrate of ammonia-magnesia mixture method (c).	Molybdic method (a).	Citrate of ammonia-magnesia mixture method (b).	Iron citrate of ammonia-magnesia mixture method (c).
A. J. Patten, Michigan	0.19	0.39	17.57	.....	24.42	.....	15.17	15.43	15.42	8.59	8.69	8.50
Wm. C. Marti, Michigan	....	....	17.92	17.57	24.70	24.47	15.28	15.20	15.32	8.73	8.37	8.53
C. van Cornell, South Carolina	.29	.45	17.54	17.15	24.50	24.84	15.36	15.75	17.40	[1]9.46	8.44	8.94
A. W. Clark, New York	.14	.21	17.98	17.88	24.67	24.58	14.78	14.62	14.54	8.32	8.00	7.45
E. G. Proulx, Indiana	.19	.26	17.85	18.41	24.75	24.81	15.10	15.31	17.52	9.08	9.10	8.64
P. L. McCreary and P. L. Hibbard, California	.21	.45	18.88	[1]22.84	25.20	25.01	[2]15.16	15.44	15.45	[2]9.17	8.78	8.76
			19.25	[1]22.38	25.36	24.98	[2]15.06	15.44	15.45	[2]9.13	8.70	8.65
L. S. Walker, Massachusetts	.23	.92	18.60	18.22	25.17	24.84	14.51	14.81	14.46	8.25	9.08	8.09
E. S. Erb, Pennsylvania	....	....	17.75	.....	24.84	.....	14.95	.....	.....	8.53	.....	.....
W. L. Whitehouse, Pennsylvania	.15	.22	17.69	17.85	24.44	24.98	14.93	.....	.....	8.38	.....	.....
L. T. Bowser, Ohio	.13	.29	18.22	18.15	24.98	24.68	15.28	15.27	17.46	8.51	8.96	8.24
Average	.19	.40	18.11	17.89	24.78	24.80	15.05	15.25	15.89	8.55	8.68	8.42

[1] Not included in average.

[2] Precipitate of magnesium pyrophosphate allowed to stand overnight, No. 2 not included in average.

TABLE 1.—*Comparative work on basic slag*—Continued.

ADDITIONAL DATA USING MODIFIED METHODS.

Modified methods used in basic slag analysis.	Sample No.	California Experiment Station.	W. L. Whitehouse, Coe-Mortimer Co.	L. T. Bowser, Dayton, Ohio.
Total phosphoric acid:				
Official *or*, volumetric method	1	17.93		
	2	24.37		
Official gravimetric method after separation of silica by dehydration	1	17.85		
	2	24.65		
Official volumetric method after separation of silica by dehydration	1	17.80		
	2	24.55		
Available phosphoric acid:				
Molybdate method, except ammonia solution was neutralized with hydrochloric acid before adding magnesia mixture. Strong ammonium hydroxid added after 15 minutes	1	15.16		
	2	8.80		
Molybdate method, except that yellow precipitate was titrated as per volumetric method	1	15.00		
	2	8.50		
Yellow precipitate was obtained by official volumetric method and determinations made volumetrically	1	15.00		
	2	8.65		
Wagner gravimetric method, except that molybdic solution and magnesia mixture were made according to the official methods	1		14.95	
	2		8.37	
Procedure as under "3" for total phosphoric acid, i. e., without separation of silica	1			15.06
	2			8.68

COMMENTS BY ANALYSTS.

E. G. Proulx, La Fayette, Ind.: In the estimation of total and available phosphoric acid several solutions were made up and determinations were made from the same solution, using different methods.

In the case of sample No. 2 the results obtained by the citrate of ammonia mixture method for total phosphoric acid agree well with those obtained by the official method, but the former apparently is not applicable with No. 1, as the final precipitate was contaminated with iron. The magnesia ammonium phosphate also precipitates slowly, requiring constant stirring for at least 20 minutes.

The molybdic method for the determination of available phosphoric acid gave concordant results. One objection to this method, however, lies in the use of 100 cc of 2 per cent ammonium hydroxid in dissolving the ammonium phosphomolybdate, as there is some danger of magnesia precipitating in a solution containing this quantity of ammonia.

The results obtained by the molybdate method and the citrate of ammonia magnesia method agree very well, although in using the latter method it required constant stirring for one hour to bring down the magnesia-ammonium phosphate.

The results obtained with the iron-citrate of ammonia-magnesia mixture method were of uncertain value. With sample No. 1 the final precipitate was contaminated with iron, which accounts for the high percentage of available phosphoric acid. Using this method it was necessary to employ ashless filter paper in order to filter off the magnesia-ammonium phosphate, as the solution would not pass through a platinum Gooch crucible. In order to obtain a pure precipitate of magnesia-ammonium phosphate the phosphoric acid in four separate aliquots from different solutions was precipitated and the double phosphate of magnesia and ammonium purified by dissolving in dilute hydrochloric acid and again precipitating by the addition of ammonium hydroxid and a few drops of magnesia mixture. This procedure resulted in final precipitates free from iron, and the results obtained agreed well with those secured by the molybdate method. In one other instance the magnesia-ammonium phosphate was purified by dissolving in dilute hydrochloric acid, neutralizing with ammonia, then acidifying with nitric acid, and completing the work according to the official method. This gave a white precipitate with result concordant with those obtained by the molybdate method. The molybdate and the citrate of ammonium-magnesia mixture methods are easy of manipulation and apparently gave correct results, while, on the other hand, the iron-citrate of ammonia-magnesia mixture method gave results of uncertain value and the method must be modified in order to be of value in the determination of available phosphoric acid in basic slag phosphates.

P. L. McCreary and P. L. Hibbard, California: In the opinion of the analysts, Messrs. P. L. McCreary and P. L. Hibbard, who did all of the work reported on, the volumetric method is much more satisfactory on basic slag than is the gravimetric method, particularly where there are quantities of soluble silica present. This is indicated in connection with subdivision 2, determination of total phosphoric acid. On comparison of the results under this subdivision you will note the close agreement between the volumetric determinations and the gravimetric determination made after dehydration and separation of silica, and also the difference between the results so obtained by the gravimetric method without the separation of silica. With regard to subdivision 3, determination of total phosphoric acid by the citrate of ammonia-magnesia mixture method, you will note the very wide discrepancy in the case of sample No. 1 between the figures obtained by this method and those obtained by method No. 2 in all its modifications. It would seem that this last method is totally inapplicable in the presence of large quantities of soluble silica. In our opinion the directions should be modified somewhat; the ammonia should be neutralized before the addition of the magnesium mixture.

W. L. Whitehouse, Coe-Mortimer Co.: In addition to the other work, I have made determinations by the Wagner method, modifying same by using the official molybdate and magnesia solutions in place of those prescribed by Wagner.

My motive in making the latter determinations was to ascertain whether or not these official solutions could be substituted for the Wagner solutions without seriously affecting the method. If this substitution did not seriously affect the method there would be eliminated the necessity of carrying the two solutions in stock. The results of this substitution you will note are highly gratifying.

L. T. Bowser, Dayton, Ohio: My results on No. 2 by the official method were not entirely satisfactory, but I could spare no more time to repeat the determination. Methods 2 and 3 check very well, it appears. With the available phosphoric acid the making of the solution went very satisfactorily, and I found no especial difficulty. Of the different methods for availability the molybdate went by far the most smoothly, was very satisfactory in fact. Method B gave rather high results on No. 2, but about the same on No. 1. Method C (iron citrate of ammonia-magnesia method) I was not very well impressed with during the course of the analysis, and the results do not check with the others. The last set (as under 3 for total phosphoric acid, without separation of silica) was not included in your list, but I ran them that way for curiosity. The results check pretty well with the others, however, and I think it is much better than Method C.

DISCUSSION OF RESULTS.

Results were received from 11 different analysts. In the determination of the total phosphoric acid the results of the various analysts, with few exceptions, agree fairly well. The average results obtained on total phosphoric acid in the basic slag by the special method (citrate of ammonia-magnesia method) are somewhat lower than when the official method was used. The special method gives results agreeing more closely with results secured after dehydration of the solution and separation of the silica; also with the volumetric method, which is not influenced by the presence of soluble silica. The two methods give results which agree fairly well with each other. The referee is of the opinion that for total phosphoric acid in slags the volumetric method should be used, and there seems to be no necessity for the recommendation of the adoption of the citrate of ammonia-magnesia method.

In the work on available phosphoric acid in both the slag and the mixture of slag and ground rock phosphate the results secured by the various analysts on the molybdate method and on the citrate of ammonia-magnesia mixture method were, as a whole, quite concordant. The two exceptions noted were due to the fact that the analyst allowed the magnesia precipitates to stand overnight before filtering instead of filtering at the end of two hours as called for by the method. The iron-citrate of ammonia-magnesia mixture method applied to the slag in some cases gave considerably higher results than the other two methods. This is probably due to iron compounds being carried down with the magnesia phosphate precipitate. Some difficulty was experienced by various analysts in getting a complete precipitation of the phosphoric acid by this method. Long continual stirring was necessary before a proper precipitation was effected. It is probable that the difficulty experienced was in a measure due to lack of familiarity with the new method.

It is worthy of note that the molybdate method modified by the use of the official volumetric method gave excellent results in the determination of available phosphoric acid in basic slags (see supplementary results by the California Experiment Station in the table). The referee did not have an opportunity, however, to test this method.

Concordant results have been secured by the so-called molybdate method in the laboratory of the Massachusetts Experiment Station, also in the Coe-Mortimer laboratory by Mr. Whitehouse, by using the ordinary molybdate solution and magnesia mixture which are mentioned in our official methods.

The results of the work on the available phosphoric acid contained in sample No. 2 show conclusively that the Wagner method is perfectly reliable in detecting the adulteration of slags by finely ground mineral phosphates.

In addition to the chemical work which has been done on basic slags during the season, the referee has made a compilation of results of field experiments with different phosphates carried on for the past 14 years at the Massachusetts Agricultural Experiment Station. These tabulated results are offered as a part of the referee's report at this time.

FIELD EXPERIMENTS MADE AT THE MASSACHUSETTS STATION TO COMPARE THE EFFICIENCY OF DIFFERENT PHOSPHATES.

DETAILED DATA.

In 1896 the field was a tough blue-grass sod and had not been plowed for many years. In April it received 600 pounds bone and 200 pounds muriate of potash per acre. After harvesting the grass the field was plowed June 24 and 25 and planted to Longfellow corn. In 1897, May 8, the field was measured off into 13 plots consisting of one-eighth acre each. After the preparation of the soil the following fertilizer schedule was applied to each plot:

Fertilizer schedule.

Ingredients.	Per plot.	Per acre.
	Pounds.	*Pounds.*
Potash-magnesia sulphate	50	400
Nitrate of soda	30¼	242
Sulphate of ammonia	12½	100

In addition to the above the various plots were fertilized as follows:

Special fertilization of various plots.

Plot.	Fertilizer.	Per plot.	Per acre.	Plot.	Fertilizer.	Per plot.	Per acre.
		Pounds.	*Pounds.*			*Pounds.*	*Pounds.*
				7	Hoof meal	11¾	94
1	Hoof meal	11¾	94	8	do	11¾	94
2	do	11¾	94		Dissolved bone black	70	560
	Apatite	32	256	9	Hoof meal	⅜	3
3	Hoof meal	11¾	94		Raw bone meal	45	360
	S. C. rock phosphate	47	376	10	Hoof meal	1 3/16	9½
4	Hoof meal	11¾	94		Dissolved bone meal	73¼	586
	Florida soft phosphate	45½	364	11	Steamed bone meal	48¼	386
5	Hoof meal	11¾	94	12	Hoof meal	11¾	94
	Basic slag	67¼	538		Acid phosphate	90½	724
6	Hoof meal	11¾	94	13	Hoof meal	11¾	94
	Navassa phosphate	49	392				

The field was planted to corn and harvested per acre as follows:

Corn harvested from plots.

Plot.	Fertilizer.	Corn per acre.	Stover per acre.	Plot.	Fertilizer.	Corn per acre.	Stover per acre.
		Bushels.	*Pounds.*			*Bushels.*	*Pounds.*
1	No phosphate	58.5	4,640	8	Dissolved bone black	61.83	4,384
2	Apatite	56.5	3,800	9	Raw bone meal	67.33	4,560
3	S. C. rock phosphate	64.5	4,280	10	Dissolved bone meal	63.33	4,400
4	Florida soft phosphate	72.5	4,960	11	Steamed bone meal	50.33	3,600
5	Phosphatic slag	62	4,960	12	Acid phosphate	62.83	4,320
6	Navassa phosphate	67.83	4,880	13	No phosphate	67.33	4,720
7	No phosphate	64.33	4,336				

In 1898 the same amount of fertilizer was applied as for the previous year and the field was planted with Fottler's Brunswick cabbage.

Cabbage harvested from plots.

Plot.	Fertilizer.	Cabbage per acre.	Plot.	Fertilizer.	Cabbage per acre.
		Pounds.			*Pounds.*
1	No phosphate	71,892	8	Dissolved bone black	42,920
2	Apatite	50,068	9	Raw bone meal	49,496
3	S. C. rock phosphate	48,840	10	Dissolved bone meal	43,160
4	Florida soft phosphate	46,120	11	Steamed bone meal	36,836
5	Phosphatic slag	47,400	12	Acid phosphate	42,440
6	Navassa phosphate	45,080	13	No phosphate	36,440
7	No phosphate	62,440			

In 1899 the fertilizer scheme was as in previous years with the exception that 50 pounds of high-grade sulphate of potash was used per plot instead of 50 pounds of potash-magnesia sulphate. The crop planted was Pride of the North corn.

Yield per acre of corn and stover, 1899.

Plot.	Fertilizer.	Shelled corn.	Stover.	Plot.	Fertilizer.	Shelled corn.	Stover.
		Bushels.	*Pounds.*			*Bushels.*	*Pounds.*
1	No phosphate	85.6	5,360	9	Raw bone meal	83.6	5,920
2	Apatite	82.0	5,520	10	Dissolved bone meal	81.1	4,160
3	S. C. rock phosphate	80.1	5,280	11	Steamed bone meal	69.5	4,000
4	Florida soft phosphate	82.1	5,600	12	Acid phosphate	68.5	4,720
5	Phosphatic slag	81.6	5,720	13	No phosphate	67.2	5,200
6	Navassa phosphate	80.3	5,640		Average of no phosphate plots	77.8	5,333.3
7	No phosphate	81.1	5,440				
8	Dissolved bone black	87.9	5,600				

In 1900 the fertilizer scheme was as in previous year. Two crops were grown, Clydesdale oats and Hungarian grass. The two crops harvested per acre as follows:

Yield per acre of oats and grass, 1900.

Plot.	Fertilizer.	Oat hay.	Hungarian hay.	Plot.	Fertilizer.	Oat hay.	Hungarian hay.
		Pounds.	*Pounds.*			*Pounds.*	*Pounds.*
1	No phosphate	5,800	3,800	8	Dissolved bone black	6,800	3,680
2	Apatite	5,880	4,000	9	Raw bone meal	6,280	3,800
3	S. C. rock phosphate	5,800	4,000	10	Dissolved bone meal	5,760	3,480
4	Florida soft phosphate	5,560	4,200	11	Steamed bone meal	5,360	3,440
5	Phosphatic slag	6,720	3,840	12	Acid phosphate	5,560	3,840
6	Navassa phosphate	5,640	3,960	13	No phosphate	3,920	3,880
7	No phosphate	5,120	3,960		Average, 1, 7, and 13	4,946.67	3,880

In 1901 fertilizers were applied, and amounts recalculated on basis of analysis to supply equal amounts of phosphoric acid and nitrogen per plot. The nitrate of soda and the potash for this year were applied so as to furnish 50 per cent more actual nitrogen and potash.

Fertilizer schedule, 1901.

Ingredients.	Per plot.	Per acre.
	Pounds.	*Pounds.*
Sulphate of potash (high grade)	37.5	300
Nitrate of soda	45.5	364
Sulphate of ammonia	12.5	100

In addition to this the various plots were fertilized as follows:

Special fertilization of various plots, 1901.

Plot.	Fertilizer.	Per plot.	Per acre.	Plot.	Fertilizer.	Per plot.	Per acre.
		Pounds.	*Pounds.*			*Pounds.*	*Pounds.*
1	Hoof meal	12.75	102	8	Hoof meal	12.75	102
2	do	12.75	102		Dissolved bone black	67.25	522
	Apatite	32	256	9	Hoof meal	2	16
3	Hoof meal	12.75	102		Raw bone	50.50	404
	S. C. rock phosphate	47	376	10	Hoof meal	1	8
4	Florida soft phosphate	45.50	364		Dissolved bone meal	54	432
	Hoof meal	12.75	102	11	Hoof meal	1.25	9
5	do	12.75	102		Steamed bone meal	47.50	380
	Phosphatic slag	67.25	538	12	Hoof meal	12.75	102
6	Hoof meal	12.75	102		Acid phosphate	47.50	380
	Tennessee phosphate	37	296	13	Hoof meal	12.75	102
7	Hoof meal	12.75	102				

Crop of Danvers Yellow Globe onions harvested, 1901.

Plot.	Fertilizer.	Onions.	Scallions.	Plot.	Fertilizer.	Onions.	Scallions.
		Bushels per acre.	*Pounds per acre.*			*Bushels per acre.*	*Pounds per acre.*
1	No phosphate	278.5	1,280	8	Dissolved bone black	209.5	600
2	Apatite	222.3	1,840	9	Raw bone	252.3	640
3	S. C. rock phosphate	235.4	1,800	10	Dissolved bone meal	213.2	600
4	Florida soft phosphate	150.6	2,280	11	Steamed bone meal	187.8	560
5	Phosphatic slag	251.8	1,160	12	Acid phosphate	187.8	920
6	Tennessee phosphate	205.7	1,720	13	No phosphate	123.4	1,800
7	No phosphate	141.4	2,000				

In 1902 the fertilizer scheme was the same as in the previous year except that 22 pounds of niter lime were added to each plot in July (176 pounds per acre). The crop grown was Danvers Yellow Globe onions.

Yield per acre of Danvers Yellow Globe onions, 1902.

Plot.	Fertilizer.	Sound onions.	Scallions.	Plot.	Fertilizer.	Sound onions.	Scallions.
		Bushels.	*Pounds.*			*Bushels.*	*Pounds*
1	No phosphate	195.7	8,560	8	Dissolved bone black	173.8	5,640
2	Apatite	101.7	8,480	9	Raw bone	301.4	4,144
3	S. C. rock phosphate	121.8	9,360	10	Dissolved bone meal	388.9	5,400
4	Florida soft phosphate	52.3	6,880	11	Steamed bone meal	243.8	5,840
5	Phosphatic slag	262	5,600	12	Acid phosphate	159.4	6,560
6	Tennessee phosphate	44.6	6,960	13	No phosphate	26.2	6,600
7	No phosphate	50.5	5,360				

In 1903 the fertilizer scheme was the same as in 1901; the crop grown was Danish Ballhead cabbage.

Yield per acre of Danish Ballhead cabbage, 1901.

Plot.	Fertilizer.	Hard heads.	Soft, leaves, stumps.	Plot.	Fertilizer.	Hard heads.	Soft, leaves, stumps.
		Pounds.	*Pounds.*			*Pounds.*	*Pounds.*
1	No phosphate	4,040	9,360	8	Dissolved bone black	8,392	31,520
2	Apatite	3,560	17,360	9	Raw bone	11,800	32,440
3	S. C. rock phosphate	12,040	24,480	10	Dissolved bone meal	12,760	29,320
4	Florida soft phosphate	3,840	21,840	11	Steamed bone meal	8,720	28,200
5	Phosphatic slag	9,920	28,040	12	Acid phosphate	6,200	24,120
6	Tennessee phosphate	1,720	29,160	13	No phosphate	440	8,080
7	No phosphate	400	14,120				

In 1904 the fertilizer scheme was the same as in the previous year except that the whole field had an application of 4,675 pounds of agricultural lime. The crop planted was Leaming field corn, which harvested as follows:

Yield per acre of Leaming field corn, 1904.

Plot.	Fertilizer.	Ensilage corn.	Plot.	Fertilizer.	Ensilage corn.
		Pounds per acre.			*Pounds per acre.*
1	No phosphate	41,000	8	Dissolved bone black	30,080
2	Apatite	40,720	9	Raw bone	45,800
3	S. C. rock phosphate	40,496	10	Dissolved bone meal	41,840
4	Florida soft phosphate	28,240	11	Steamed bone meal	28,400
5	Phosphatic slag	36,440	12	Acid phosphate	29,040
6	Tennessee phosphate	32,120	13	No phosphate	20,240
7	No phosphate	32,344			

In 1905 the fertilizer scheme was the same as in the previous year. The crop grown was grass, timothy, red top, alsike clover, common red clover, mammoth red clover. The crop was harvested August 21 and was mostly weeds; not weighed.

In 1906 the fertilizer applied was the same as in the previous year except that plot 2 received no apatite. A crop of hay and rowen was harvested with the following results:

Yield per acre of hay and rowen, 1906.

Plot.	Fertilizer.	Hay.	Rowen.	Plot.	Fertilizer.	Hay.	Rowen.
		Pounds.	*Pounds.*			*Pounds.*	*Pounds.*
1	No phosphate	6,600	1,800	7	No phosphate	7,000	2,200
2	Apatite (none this year)	7,520	1,720	8	Dissolved bone black	6,960	2,240
3	S. C. rock phosphate	7,440	1,616	9	Raw bone	7,120	2,000
4	Florida soft phosphate	7,600	1,504	10	Dissolved bone meal	7,520	2,552
5	Phosphatic slag	7,600	1,600	11	Steamed bone meal	7,120	2,264
6	Tennessee phosphate	7,120	1,624	12	Acid phosphate	7,080	2,320
				13	No phosphate	6,560	1,600

In 1907 the fertilizer was applied as in previous year except that plot 2 received its usual quantity of apatite, which was omitted during the previous year. Mixed grasses and clover constituted the crop, which harvested as follows:

Yield per acre of hay and rowen, 1907.

Plot.	Fertilizer.	Hay.	Rowen.	Plot.	Fertilizer.	Hay.	Rowen.
		Pounds.	*Pounds.*			*Pounds.*	*Pounds.*
1	No phosphate	8,400	400	8	Dissolved bone black	9,200	488
2	Apatite	8,800	504	9	Raw bone	9,240	504
3	S. C. rock phosphate	8,480	496	10	Dissolved bone meal	9,160	504
4	Florida soft phosphate	8,480	400	11	Steamed bone meal	8,320	560
5	Phosphatic slag	8,360	416	12	Acid phosphate	8,040	600
6	Tennessee phosphate	8,040	328	13	No phosphate	7,240	360
7	No phosphate	8,160	240				

In 1908 the fertilizer schedule was the same as in the previous year except that plot 2 received 47 pounds of Arkansas rock phosphate in place of 32 pounds of apatite, as in the previous schedule. Cabbage was grown and harvested as follows:

Yield of cabbage, 1908.

Plot.	Fertilizer.	Cabbage.	Plot.	Fertilizer.	Cabbage.
		Pounds.			*Pounds.*
1	No phosphate	3,800	8	Dissolved bone black	20,080
2	Arkansas rock phosphate	4,480	9	Raw bone	20,240
3	S. C. rock phosphate	11,440	10	Dissolved bone meal	12,320
4	Florida soft phosphate	10,720	11	Steamed bone meal	11,440
5	Phosphatic slag	19,120	12	Acid phosphate	8,280
6	Tennessee phosphate	5,600	13	No phosphate	1,840
7	No phosphate	2,080			

In 1909 the fertilizer schedule was the same as in the previous year; the crop grown was medium early yellow soy bean.

Yield per acre of yellow soy beans, 1909.

Plot.	Fertilizer.	Beans.	Straw.	Plot.	Fertilizer.	Beans.	Straw.
		Pounds.	*Pounds.*			*Pounds.*	*Pounds.*
1	No phosphate	1,796	3,220	7	No phosphate	1,712	2,272
2	Arkansas rock phosphate	1,744	3,176	8	Dissolved bone black	1,672	2,968
				9	Raw bone	1,856	3,088
3	S. C. rock phosphate	1,816	3,384	10	Dissolved bone meal	1,908	2,892
4	Florida soft phosphate	1,836	3,124	11	Steamed bone meal	1,964	2,676
5	Phosphatic slag	1,840	3,264	12	Acid phosphate	1,904	2,552
6	Tennessee phosphate	1,812	2,620	13	No phosphate	1,496	1,784

In 1910 the fertilizer schedule was the same as in the previous year; the crop grown was potatoes, which harvested as follows:

Yield of potatoes, 1910.

Plot.	Fertilizer.	Merchantable potatoes.	Plot.	Fertilizer.	Merchantable potatoes.
		Bushels per acre.			*Bushels per acre.*
1	No phosphate	286.4	8	Dissolved bone black	247.9
2	Arkansas rock phosphate	289.3	9	Raw bone meal	258.8
3	S. C. rock phosphate	264.8	10	Dissolved bone meal	264.3
4	Florida soft phosphate	234.8	11	Steamed bone meal	261.9
5	Phosphatic slag	245.5	12	Acid phosphate	257.2
6	Tennessee phosphate	236.4	13	No phosphate	214.7
7	No phosphate	244.1			

SUMMARY.

Using the average of plots 7 and 13 (no-phosphate plots) as the basis of comparison, and designating the average yield on these plots as 100, the following table No. 1 will show the comparative efficiency of acid phosphate, basic slag phosphate, and dissolved bone black as sources of available phosphoric acid for the crops mentioned.

SUMMARY No. 1.—*Comparative efficiency of phosphates and bone black based on "no-phosphate" plot as 100.*

Year.	Crop.	No phosphate.	Acid phosphate.	Basic slag phosphate.	Dissolved bone black.
1897	Corn	100	95	94	94
	Stover	100	95	110	97
1898	Cabbage	100	86	96	87
1899	Corn	100	92	110	118
	Stover	100	89	108	105
1900	Oat Hay	100	123	149	150
	Hungarian hay	100	98	98	94
1901	Onions	100	142	190	158
	Scallions	100	48	61	32
1902	Onions	100	415	656	453
	Scallions	100	110	94	94
1903	Cabbage	100	263	330	346
1904	Ensilage corn	100	110	139	114
1905	Mixed grasses largely weeds				
1906	Hay	100	104	112	108
	Rowen	100	122	84	118
1907	Hay	100	104	109	119
	Rowen	100	200	139	163
1908	Cabbage	100	422	976	1,024
1909	Soy beans	100	119	115	104
	Bean straw	100	125	161	146
1910	Potatoes	100	112	107	108
	Average for 14 years	100	157	219	212

Using the average yield with acid phosphate and dissolved bone black as a standard, and designating the same as 100, the following table will show more graphically the value of the basic slag phosphate as compared with the two dissolved phosphates as a source of available phosphoric acid.

SUMMARY No. 2.—*Efficiency of basic phosphate as compared with the average of acid phosphate and bone black.*

Year.	Crop.	Average of acid phosphate and dissolved bone black.	Basic slag phosphate.
1897	Corn	100	99
	Stover	100	115
1898	Cabbage	100	111
1899	Corn	100	105
	Stover	100	111
1900	Oat hay	100	109
	Hungarian hay	100	102
1901	Onions	100	127
	Scallions	100	153
1902	Onions	100	151
	Scallions	100	92
1903	Cabbage	100	108
1904	Ensilage corn	100	124
1905	Mixed grasses, largely weeds		
1906	Hay	100	108
	Rowen	100	70
1907	Hay	100	98
	Rowen	100	77
1908	Cabbage	100	135
1909	Soy beans	100	103
	Bean straw	100	119
1910	Potatoes	100	97
	Average for 14 years	100	111

Considered individually some of the field experiments have not been very positive in showing the efficiency of the phosphoric acid which the various phosphates furnish. The no-phosphate plots in some instances have furnished as good and even better yields than many of the phosphate plots. Especially is this true in the case of plot No. 1, which was used as a farm garden for some years prior to its selection as part of the field for the phosphate experiments. The longer the fields are used for the experiments the better are the conditions for obtaining comparative results, and during the more recent years the yields have been more significant in indicating the efficiency of the phosphoric acid from various sources.

Particular attention is called to the summaries. It will be noted that for only 3 years out of the 14 has the efficiency of basic slag been less than the acid phosphate, and in the average for 14 years with nine different crops it has proved superior as a source of available phosphoric acid to both acid phosphate and dissolved bone black. It seems to the referee that these field experiments, conducted in a careful, scientific way and covering a period of 14 years, furnish reliable evidence of the high availability of the phosphoric acid in basic slag phosphate and emphasize the importance of the adoption of some chemical method which will give full credit to the phosphoric acid contained in this material. The field experiments will undoubtedly be continued, and I hope will serve in the final report of the special committee and in their recommendations as to the final adoption of the Wagner method as official by the association during the year 1914.

CONCLUSIONS.

The results of cooperative chemical work show the Wagner method to be reliable for the detection of adulteration of basic slag. Long-continued field experiments, as well as the Wagner method of analysis, show the uniform availability of the phosphoric acid which a bona fide basic slag carries, and taken together the analytical results strongly emphasize the importance of the provisional adoption by the association of the Wagner method of analysis.

It is therefore recommended—

(1) That the following method of analysis be adopted provisionally by this association pending further study and more complete data in regard to field and pot experiments.

A. *Making the citric solution.*—Weigh 5 grams of the basic slag; transfer to a one-half-liter Wagner flask containing 5 cc of 95 per cent alcohol. The flask should have a neck width of at least 20 mm and be marked at least 8 cm below the mouth. Make up to the mark with dilute citric acid solution (2 per cent) of a temperature of 17.5° C. Fit the flask with a rubber stopper and put at once into the rotary apparatus for 30 minutes, making 30 to 40 revolutions per minute. Take off and filter immediately.

B. *Analysis of the citric solution.*—As soon as the filtration is completed analyze the solution at once according to the so-called molybdate method, which in detail is as follows: To 50 cc of the clear filtrate add 100 cc of molybdate solution made according to the official methods. Keep the beaker in a water bath until the temperature reaches 65° C.; take out and allow to cool at ordinary temperature. Then filter and wash the yellow precipitate of phosphomolybdate of ammonia four or five times with 1 per cent nitric acid. Dissolve in 100 cc of 2 per cent ammonium hydroxid (cold), nearly neutralize with hydrochloric acid, and add to the solution 15 cc of magnesia mixture (made according to the official method) drop by drop during continuous stirring. After 15 minutes add from 10 to 12 cc of ammonium hydroxid solution (specific gravity 0.90), then cover the beaker with a glass cover and allow to stand for about two hours. Filter the double phosphate of ammonia and magnesia through a tared platinum Gooch crucible, wash six times with 2 per cent ammonium hydroxid, dry, and proceed as is customary for phosphoric acid determinations.

(2) That further work be done with the citrate of ammonia-magnesia mixture method and the official volumetric method, using the Wagner method of making the citric solution of the slag. The referee believes that the official volumetric method will prove a very satisfactory one for the analysis of the citric solution; sufficient work, however, has not been done to warrant its adoption at this time even as a provisional method.

NEUTRAL AMMONIUM-CITRATE SOLUTION.

By A. J. PATTEN and C. S. ROBINSON.

Since the proposal of the ammonium-citrate method for the determination of available phosphoric acid, much trouble has been experienced in preparing a strictly neutral solution of the reagent. The weakness of both the acid and the base renders the end point quite indistinct with ordinary indicators, and much time and patience are required on the part of the operator to obtain the desired results. Several modifications of the simple titration method have been proposed, but each has objections which prohibit its common acceptation by practical chemists. The importance which the method has assumed in agricultural work demands, however, that some convenient means be devised for preparing the necessary solution. Such a method has recently been proposed by Hall and Bell[1] and was later shown by Hall[2] to be quite suitable for ordinary laboratory use. At the time these articles appeared the authors of this paper were engaged in working out the same method, and the results are here offered, not with the idea of claiming any credit for the development of the method, but simply as corroborative evidence in favor of its general acceptation.

The method of procedure is essentially the same as that followed by Hall. A solution of citric acid was almost neutralized, care being taken to keep the density above 1.09. Small samples of this solution were then titrated with a dilute solution of ammonium hydroxid (about 3 per cent) to determine the approximate amount required to neutralize the remaining acid. Definite quantities of the citrate solution were then removed with a pipette and transferred to clean, dry flasks. To these portions of the original solution varying quantities of the dilute ammonia solution were added in such a way that several contained more and several less than the approximate amount required for exact neutralization, as determined by the titration with corallin. These solutions were then made up to a definite volume and placed in a thermostat, the temperature of which was held constant, and allowed to come to the temperature of the bath, after which their resistances were measured by the Wheatstone bridge method. Plotting the cubic centimeters of ammonium hydroxid added against the bridge readings gave a curve from which could be read the amount of ammonia required to neutralize the acid remaining in a given quantity of the citrate solution. Only one of several titrations by this method is here shown:

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[1] J. Amer. Chem. Soc., 1911, **33**: 711.

[2] J. Ind. Eng. Chem., 1911, **3**: 559.

Titration curve.

[Temperature, 33.3° C. ±0.02. Resistance in box, 5 ohms.]

	Bridge reading.	Ammonium hydroxid.		Bridge reading.	Ammonium hydroxid.
		cc per 100 cc sol.			*cc per 100 cc sol.*
No. 1	582.25	0.0	No. 5	588.50	20.0
No. 2	585.25	5.0	No. 6	585.75	25.0
No. 3	588.50	10.0	Neutral point calculated	590.75	15.0
No. 4	590.75	15.0	Neutral point observed	590.75	15.0

In order to determine the personal factor in making up the solution by the other methods of determining the neutral point, portions of the citric acid solution were neutralized by the corallin and the purified litmus methods by four laboratory assistants, working independently. The dilute ammonia solution used was kept in a burette inclosed in opaque paper to prevent the reading being taken until the supposed neutral point had been reached. In this way each operation was made independent of the others. Great care was taken that no loss of ammonia occurred during the process. The results are given in the following table:

Amount of dilute ammonia solution used per 100 cc citric acid solution, by four different observers.

Number.	Ammonium hydroxid.	Number.	Ammonium hydroxid.
	cc per 100 cc sol.		*cc per 100 cc sol.*
Corallin used as indicator:		Corallin used as indicator:	
P-I	12.30	M-I	16.00
P-II	12.10	M-II	15.00
P-III	16.20	M-III	16.40
P-IV	12.70	M-IV	16.60
R-I	12.00	I-I	12.20
R-II	12.00	I-II	13.60
R-III	14.30	I-III	19.60
R-IV	13.60	I-IV	15.90
Purified litmus as indicator:		Purified litmus as indicator:	
P-I	15.00	M-I	14.00
P-II	13.76	M-II	15.00
P-III	16.00	M-III	12.50
R-I	15.00	I-I	15.82
R-II	17.50	I-II	15.82
R-III	14.00	I-III	15.00

The conductivity of some of the solutions was determined and the readings found to fall on the curve at the points to be expected from the quantities of alkali used. The amounts of ammonium hydroxid vary considerably, and in only one case, using corallin, was the exact neutral solution obtained, while with the litmus method one-third of the trials gave a neutral solution. These examples fairly illustrate the difficulty of making an exactly neutral reagent by the methods most commonly in use, and it is quite possible that in many cases the character of the neutral ammonium-citrate solution varies more than in the cases cited, and that considerable error may be introduced in this way.

To ascertain whether or not these differences in the citrate solution would produce marked difference in the results of analyses made with them, several actual determinations were made. The solutions used were R-II, C, M-IV, and I-III. (See preceding table.) Solutions were carefully diluted to a specific gravity of 1.09 and the determinations were all made at one time so that there could be no variation due to temperature of bath. Determinations were made on two samples of fertilizer, one containing a small percentage of insoluble phosphoric acid and the other a large percentage. The results are given in the following table:

Determinations made to test effect of differences in the citrate solution.

Solution.	Ammonium hydroxid.	Per cent of phosphoric acid.	
		Sample 1.	Sample 2.
	cc per 50 cc sol.		
R-II	6.0	2.84 2.81 2.83	8.86 8.82
C	7.5	3.74 3.54 3.80	9.80 9.82
M-IV	8.3	4.23 4.07 4.21	10.10 9.96
I-III	9.8	4.79 4.77 4.90	10.56 10.36

REPORT ON NITROGEN.

By James W. Kellogg, *Referee.*

At the last meeting committee A recommended that the referee be requested to study laboratory methods for the determination of availability of organic nitrogen. Seven samples were prepared and sent to about 25 chemists who had signified their willingness to take part in the cooperative work. Three methods were suggested to be used, one being the pepsin hydrochloric acid solution method, and the other two the provisional methods outlined in Bulletin 107, Revised, namely, the alkaline permanganate method and the neutral permanganate method. Some time after the samples and methods had been sent out, my attention was called to the fact that the provisional methods had been modified and a large amount of work had been done with these modified methods. This information was received too late to be sent to the analysts taking part, or to change the experiments already begun by suggesting the use of the later methods. Only a few analysts reported results obtained by the methods suggested by the referee, and several called attention to recent modifications of the same. Owing to the late date at which some of these reports were received, and because the methods suggested for trial are not now being used by those who have given this subject more recent study, it did not appear to be advisable to report the same to the association. The alkaline permanganate method, as modified by Jones, has been adopted by the experiment stations of New York, New Jersey, and the New England States, and the neutral permanganate method as modified by Street is being used by some southern analysts.

Inasmuch as the modified methods are receiving more attention than the methods suggested for the year's work, further consideration of the few results obtained did not seem advisable.

It is recommended, therefore, that the study of methods for the determination of available nitrogen be continued and that the alkaline permanganate and the neutral permanganate methods as now used be considered as applied to crude stock and to commercial fertilizers.

A resolution was introduced by E. L. Baker to the effect that the following method for the determination of nitrogen in commercial nitrates be referred to the referee for 1912 for trial, and the resolution was adopted:

Determination of nitric and nitrous nitrogen (Salle, Ann. chim. anal., 1910, 15: 103–105).—To 0.5 gram of the nitrates in a 600–700 cc flask add 200 cc of distilled water, 5 grams of powdered zinc, from 1 to 2 grams of ferrous sulphate, and 50 cc of a 36° Baumé soda solution. In the neck of the flask place some glass wool and connect with the distilling apparatus. Distil off the ammonia and collect as usual in decinormal sulphuric acid and titrate.

REPORT ON POTASH.

By E. L. BAKER, *Referee.*

The potash work this year has been a repetition of that of the past year, with only minor changes in the methods. In order to test thoroughly the applicability of the proposed methods to different materials, three samples were used representing high-grade salts, kainits, and mixed fertilizers. Sample No. 3 was made by mixing weighed amounts of acid phosphate and muriate and contained, theoretically, 4.85 per cent of potash. The following directions were sent to cooperating chemists:

INSTRUCTIONS FOR POTASH WORK, 1911.

Sample No. 1, commercial muriate; sample No. 2, kainit; sample No. 3, acid phosphate and potash. Thoroughly mix samples before use. Potash in each sample to be determined by the official method (Bul. 107, Rev., Bureau of Chemistry) and by the volumetric and gravimetric cobalti-nitrite methods.

VOLUMETRIC METHOD (DRUSHEL'S SLIGHTLY MODIFIED).[1]

Sodium nitrite solutions.—Dissolve 220 grams of sodium nitrite in water and dilute to 500 cc.

Cobalt acetate solution.—Dissolve 113 grams of cobalt acetate in about 300 cc of water, add 100 cc of glacial acetic acid, and dilute to 500 cc.

Sodium cobalti-nitrite solution.—Mix equal parts of the sodium nitrite and cobalt acetate solutions a few hours before required for use. A yellow precipitate forms on standing. Filter just before using.

Standard solutions.—Fifth-normal potassium permanganate and fifth-normal oxalic acid; solutions of fertilizers to be made up according to the official method.

Sample No. 1.—Run 10 cc of solution, equal to one-fifth of a gram, from a burette into a porcelain evaporating dish; dilute with about 25 cc of water and add 1 cc of glacial acetic acid; then add slowly, so that the precipitate may not be too finely divided, 15 cc of sodium cobalti-nitrite reagent; evaporate on a steam or water bath to a thick sirup, which becomes just firm on cooling. Care should be taken not to evaporate to dryness. Stir with cold water until excess of cobalti-nitrite reagent has dissolved; allow to settle and decant two or three times through a small funnel containing a perforated porcelain disk and a thick pad of ignited asbestos. Transfer precipitate to filter and wash thoroughly with water.

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[1] Chem. News, 1908, 97: 124.

In the meantime an excess of fifth-normal potassium permanganate (about 65 cc) is measured into a 500 cc Erlenmeyer flask, diluted with about 200 cc of water and heated on a hot plate nearly to boiling. To this solution transfer the precipitate and asbestos; mix well by revolving the contents of the flask and maintain at nearly a boiling temperature, stirring frequently. In a few minutes manganese hydroxid separates out and the solution darkens; continue heating with occasional stirring 10 to 15 minutes longer, or until the color of the permanganate disappears and the supernatant liquid becomes clear; then add 5 cc of dilute sulphuric acid (1 to 1), stir, and allow to stand about 5 minutes in order to oxidize the last traces of the potash precipitate; add an excess of fifth-normal oxalic acid (15 cc); keep the solution hot until it becomes colorless and the manganese hydroxid precipitate has completely dissolved, then titrate to color with fifth-normal potassium permanganate.

From the whole amount of permanganate employed, subtract the permanganate equivalent of the oxalic acid used and multiply the remainder by the factor 0.001712, which is the factor for strictly fifth-normal potassium permanganate.

GRAVIMETRIC COBALTI-NITRITE METHOD.

Sample No. 1.—Precipitate precisely as above. Filter through a Gooch crucible, wash thoroughly with cold water and finally four or five times with 80 per cent alcohol. Dry one hour, or until constant weight is obtained, in a steam or water oven.

Multiply weight by the factor 0.2075. Report time required to obtain constant weight.

Sample No. 2.—Measure 25 cc of solution, equivalent to 0.5 gram, into a porcelain evaporating dish, add 1 cc of glacial acetic acid and 10 cc of cobalti-nitrite reagent, and determine potash by the volumetric and gravimetric cobalti-nitrite methods as in sample No. 1, using about 45 cc of fifth-normal potassium permanganate and 15 cc of fifth-normal oxalic acid.

Sample No. 3.—(a) Measure 50 cc of solution into a platinum evaporating dish, add sulphuric acid (1:1) and ignite as in the official method. Transfer to a porcelain evaporating dish with hot water and concentrate to a volume of 5 to 10 cc. Add 1 cc of glacial acetic acid and 10 cc of reagent. Proceed as in sample No. 1, using about 30 cc of permanganate and 15 cc of oxalic acid. (Note. In making up the solution of this sample care should be taken to filter at once after precipitating with ammonium hydroxid and ammonium oxalate and cooling, as long standing of the solution in the presence of the insoluble matter seems to produce higher results.)

(b) Determine potash also by the gravimetric cobalti-nitrite method, using 50 cc aliquot, and by the offical method.

MODIFIED OFFICIAL METHOD.

Sample No. 3.—Weigh 2.5 grams upon a 12.5 cm filter paper and wash with successive small portions of boiling water into a 250 cc graduated flask, to a volume of about 200 cc; add 2 cc of concentrated hydrochloric acid, heat to boiling, and precipitate with ammonium hydroxid and ammonium oxalate; cool, dilute to 250 cc, filter, take a 50 cc aliquot equivalent to 0.5 gram, and finish by the official method.

(Note.—Corrections should be made and reported for blanks upon all determinations.)

REMARKS.

The accuracy of the volumetric method depends mainly upon two things: The method of precipitation and the oxidation of the potash precipitate during titration.

To obtain complete precipitation a liberal excess of reagent must be used and strict adherence to the directions for evaporation should be observed. In the case of mixed fertilizers, if the solution is too dilute when the reagent is added, a very finely divided precipitate forms, which is hard to handle. With potash salts the same seems to be true if the solution is too concentrated.

In the titration the oxidation must be complete before oxalic acid is added. The darkening of the permanganate solution is no sign that the yellow precipitate is entirely decomposed, for it requires from 5 to 15 minutes longer time, depending upon the amount of precipitate present. The disappearance of the color of the permanganate is a fairly good indication that the reaction is completed, although it is well to examine the surface of the solution closely for traces of the yellow precipitate, which can usually be seen if present.

COMPARISON OF THE OFFICIAL, THE MODIFIED OFFICIAL, AND THE VOLUMETRIC AND GRAVIMETRIC COBALTI-NITRITE METHODS.

Cooperative results on potash (percentage).

Analyst.	Sample No. 1.			Sample No. 2.			Sample No. 3.			
	Official method.	Cobalti-nitrite.		Official method.	Cobalti-nitrite.		Official method.	Cobalti-nitrite.		Modified official method.
		Volumetric.	Gravimetric.		Volumetric.	Gravimetric.		Volumetric.	Gravimetric.	
H. H. Hill, Blacksburg, Va.	51.37	51.27	[1]51.33	13.19	13.28	13.23	4.49	4.61	4.63	4.67
	51.38	[1]51.36	51.50	13.20	[1]13.20	13.31	4.51	4.60	4.77	4.70
	....	51.44	[1]51.35	13.21	....	....	....	[1]4.56	[1]4.52	4.73
Average.......	51.38	51.36	51.39	13.20	13.24	13.27	4.50	4.59	4.64	4.70
P. L. Hibbard, Berkeley, Cal.	....	48.93	48.20	....	12.40	12.95	....	4.51	5.32	4.80
	....	48.93	49.80	....	12.57	13.00	....	4.53	5.39	4.76
	....	49.01	....	....	12.70	....	....	4.64	....	....
	....	48.50	....	....	....	....	....	4.56	....	....
	....	48.93	....	....	....	....	....	4.51	....	....
	....	49.27	....	....	....	....	....	....	....	....
	....	50.15	....	....	....	....	....	....	....	....
	....	49.43	....	....	....	....	....	....	....	....
	....	48.66	....	....	....	....	....	....	....	....
	....	50.54	....	....	....	....	....	....	....	....
Average.......	....	49.24	49.00	....	12.56	12.98	....	4.55	5.36	4.78
P. L. McCreary, Berkeley, Cal.	51.40	49.20	50.68	13.26	....	13.65	4.55	4.40	....	4.70
	51.44	49.30	51.30	13.28	....	....	4.58	4.40	....	4.72
	....	49.60	....	....	....	....	....	4.40	....	....
	....	50.20	....	....	....	....	....	....	....	....
	....	50.46	....	....	....	....	....	....	....	....
	....	50.63	....	....	....	....	....	....	....	....
	....	51.48	....	....	....	....	....	....	....	....
Average.......	51.42	50.12	50.99	13.25	....	13.65	4.57	4.40	....	4.71
O. M. Shedd, Lexington, Ky.	51.65	48.83	52.18	13.37	12.70	13.48	4.52	4.53	4.48	....
	51.57	48.54	52.40	13.40	12.68	13.51	4.53	4.51	4.45	....
	....	50.82	....	....	13.00	....	....	....	....	....
	....	50.12	....	....	12.99	....	....	....	....	....
Average.......	51.61	49.58	52.29	13.39	12.84	13.50	4.53	4.52	4.47	....
Arao Itano, East Lansing, Mich.	51.60	51.19	51.77	13.64	13.01	13.04	4.67	4.41	6.27	....
	51.60	50.85	51.92	13.60	13.05	13.14	4.77	4.47	6.26	....
	51.67	50.20	51.25	13.73	13.01	13.49	4.69	4.37	6.37	....
	51.48	49.91	51.12	13.72	12.98	13.51	....	4.30	6.29	....
	....	....	....	....	....	13.51	....	4.38	7.22	....
	....	....	....	....	....	13.51	....	4.44	7.44	....
	....	....	....	....	....	....	....	....	7.42	....
	....	....	....	....	....	....	....	....	7.33	....
Average.......	51.58	50.50	51.51	13.67	13.01	13.36	4.71	4.39	6.82	....
Cornelius Beatty, College Park, Md.	51.75	....	51.23	13.18	10.85	13.15	4.69	3.49	4.61	4.69
	51.85	....	51.62	13.22	11.06	13.24	4.73	5.17	4.61	4.69
	52.35	....	51.85	13.23	11.23	13.30	....	5.51	....	4.71
	52.40	....	52.00	13.28	11.30	13.32	....	5.70	....	4.73
	....	....	52.14	....	11.30	13.32	....	....	....	....
	....	....	52.20	....	11.36	13.35	....	....	....	....
	....	....	52.31	....	11.39	13.37	....	....	....	....
	....	....	52.36	....	11.47	13.42	....	....	....	....
Average.......	52.09	....	51.96	13.23	11.25	13.31	4.71	4.97	4.61	4.71

[1] Used porous clay crucibles in filtering.

Cooperative results on potash (*percentage*)—Continued.

Analyst.	Sample No. 1.			Sample No. 2.			Sample No. 3.			
	Official method.	Cobalti-nitrite.		Official method.	Cobalti-nitrite.		Official method.	Cobalti-nitrite.		Modified official method.
		Volumetric.	Gravimetric.		Volumetric.	Gravimetric.		Volumetric.	Gravimetric.	
L. T. Bowser, Dayton, Ohio.	50.80	51.74	50.90	13.09	13.44	13.31	4.58	4.65	6.11	4.82
	51.16	51.14	51.29	12.98	13.51	13.26	4.55	4.74	5.84	....
	....	51.05	50.20	13.29	13.34	13.29	....	4.65	5.69	....
	....	51.23	50.90	....	13.30	13.39	....	4.40	5.91	....
	....	51.56	50.57	....	13.44	13.31	....	4.51	5.79	....
	....	50.79	50.80	....	13.44	13.24	....	4.52	5.61	....
Average........	50.98	51.25	50.78	13.12	13.41	13.30	4.56	4.58	5.83	4.82
A. H. Allen, Richmond, Va.	51.36	....	....	13.52	....	....	4.73	....	....	....
	51.44	....	....	13.48	....	....	4.71	....	....	....
Average........	51.40	....	....	13.50	....	....	4.72	....	....	....
F. D. Fuller and J. H. Roop, La Fayette, Ind.	51.37	48.12	51.80	13.31	12.11	11.84	4.61	4.48	6.71	4.55
	51.35	....	....	13.21	11.80	11.75	4.63	4.45	6.95	4.70
	....	....	....	....	....	....	....	4.40	6.86	....
	....	....	....	....	....	....	....	4.42	....	....
Average........	51.36	48.12	51.80	13.26	11.96	11.80	4.62	4.44	6.84	4.63
H. D. Edmond, Storrs, Conn.	51.59	49.86	51.83	13.41	13.06	13.64	4.72	4.59	4.75	4.83
	51.65	49.48	51.49	13.38	12.98	13.67	4.77	....	4.68	4.90
Average........	51.62	49.67	51.66	13.40	13.02	13.66	4.75	4.59	4.72	4.87
J. M. Bartlett and A. G. Durgin, Orono, Me.	52.12	51.57	50.38	13.24	14.74	13.23	4.79	....	4.74	4.64
	52.00	51.32	50.26	13.53	14.71	13.22	4.70	....	4.73	4.52
	51.84	53.46	51.54	13.36	14.26	13.29	4.72	....	4.73	4.52
	51.72	52.00	52.35	13.56	....	13.60	4.65	....	4.80	....
Average........	51.92	52.09	51.13	13.42	14.57	13.34	4.72	....	4.75	4.56
J. C. Jurjenns, Madison, Wis., average.	50.78	50.37	50.50	13.53	13.34	13.52	4.78	4.21	6.21	[1] 4.25
G. F. Lipscomb, Clemson College, S. C.[2]	51.17	51.00	51.91	13.43	13.12	14.05	5.14	5.12	5.30	....
	....	51.25	52.07	....	12.88	14.47	....	5.02	5.08	....
	....	51.59	51.90	....	12.88	13.91	....	5.12	5.36	....
	....	51.25	50.97	....	13.16	13.65	....	5.12	....	....
	....	51.25	51.01	....	13.02	13.60	....	5.40	....	....
	....	....	51.57	....	13.02	13.94	....	5.12	....	....
	....	....	....	....	13.17	13.65	....	....	....	....
	....	....	....	....	....	13.95	....	....	....	....
Average........	51.17	51.27	51.57	13.43	13.04	13.90	5.14	5.15	5.25	....
Thos. C. Pinkerton, Philadelphia, Pa.	51.51	49.73	49.39	13.31	12.70	12.07	4.49	4.40	5.25	4.62
	51.41	49.78	49.18	13.29	12.60	12.12	4.56	4.54	5.20	4.54
	51.22	....	49.30	....	....	12.37	4.60	....	5.44	....
	....	....	....	....	....	....	....	....	5.38	....
Average........	51.38	49.76	49.29	13.30	12.65	12.19	4.55	4.47	5.32	4.58
R. M. Pinckney, Boseman, Mont.	51.72	47.96	48.09	15.18	10.55	10.85	5.02	4.54	6.94	4.94
	51.50	47.08	49.28	14.97	11.09	10.94	4.81	4.54	6.66	4.82
	....	47.08	50.09	....	10.37	10.81	....	4.50	6.62	....
	....	....	49.16	....	10.75	....	....	4.41	6.97	....
	....	....	51.36	....	10.92	....	....	4.45	6.94	....
Average........	51.61	47.37	49.60	15.08	10.74	10.87	4.92	4.49	6.83	4.88
F. B. Porter, Atlanta, Ga.	52.08	51.53	50.83	13.30	12.72	12.14	4.54	4.61	4.74	4.55
	52.06	50.57	50.99	13.44	12.62	12.20	4.57	4.58	4.87	4.59
	52.14	51.36	50.26	13.38	12.39	12.16	4.55	4.61	4.48	4.57
	....	50.42	51.19	....	12.39	12.18	....	....	....	....
	....	50.50	50.39	....	13.38	12.16	....	....	....	....
	....	50.93	51.34	....	....	....	....	....	....	....
Average........	52.09	50.89	50.83	13.37	12.70	12.17	4.55	4.60	4.70	4.57

[1] Omitted from general average.

[2] Work done by two students under supervision of G. F. Lipscomb.

Cooperative results on potash (percentage)—Continued.

Analyst.	Sample No. 1.			Sample No. 2.			Sample No. 3.			
	Official method.	Cobalti-nitrite.		Official method.	Cobalti-nitrite.		Official method.	Cobalti-nitrite.		Modified official method.
		Volumetric.	Gravimetric.		Volumetric.	Gravimetric.		Volumetric.	Gravimetric.	
Reported by Paul Rudnick: W. D. Turner, Chicago, Ill.	....	....	....	....	....	....	....	....	5.10	....
	....	....	....	....	....	....	....	....	5.16	....
	....	....	....	....	....	....	4.56	....	5.06	....
	....	....	....	....	....	....	4.58	....	5.12	....
	52.15	51.99	52.29	13.60	13.26	13.78	4.56	4.51	5.18	....
	52.09	51.90	52.21	13.76	13.34	13.64	4.59	4.49	5.45	....
Average.......	52.12	51.95	52.26	13.68	13.30	13.71	4.57	4.50	5.12	....
E. E. Elm, Chicago, Ill.	51.57	51.96	52.19	13.70	13.27	13.63	4.62	4.41	4.96	....
	51.66	51.45	52.22	13.87	13.34	13.50	4.55	4.24	4.86	4.74
	51.59	51.75	52.65	....	....	....	....	4.48	4.73	4.88
	51.42	51.83	52.65	....	....	....	....	4.40	[1] 5.21	....
Average.......	51.56	51.75	52.43	13.78	13.31	13.57	4.59	4.38	4.84	4.81
L. S. Walker, Amherst, Mass.	51.36	49.88	51.88	13.37	13.04	13.58	4.69	4.58	4.70	4.75
	51.44	50.40	52.21	13.32	13.04	....	4.77	4.41	4.85	4.75
Average.......	51.40	50.14	52.05	13.35	13.04	13.58	4.73	4.50	4.78	4.75
A. G. Hogan and L. E. Morgan, Columbia, Mo.	51.50	47.60	51.00	12.93	13.17	13.22	4.69	4.19	5.08	4.63
	51.30	49.20	51.35	13.07	13.63	13.30	4.63	4.42	4.72	4.61
	51.55	47.45	....	13.02	13.34	13.66	4.72	4.06	6.18	....
	....	48.34	....	....	13.76	....	....	....	....	....
	....	48.21	....	....	13.64	....	....	....	....	....
	....	49.02	....	....	....	....	....	....	....	....
Average	51.45	48.30	51.18	13.01	13.51	13.40	4.68	4.22	5.32	4.62
M. P. Sweeney, Geneva, N. Y.	52.26	52.41	52.34	13.24	13.72	12.49	4.59	4.64	4.71	4.62
	52.16	52.47	52.27	13.68	13.89	12.87	4.53	4.81	4.71	4.68
	52.31	52.29	52.09	13.37	13.27	13.50	4.57	4.88	4.68	4.67
	52.28	52.29	52.22	13.44	13.33	12.76	....	....	4.59	....
	....	52.37	....	....	....	12.91	....	....	....	....
Average.......	52.26	52.36	52.25	13.44	13.55	12.85	4.56	4.73	4.68	4.65
H. P. Fishburn, Moscow, Idaho.	51.86	49.47	49.18	13.84	12.61	13.12	4.78	4.51	4.72	....
	51.86	48.95	49.13	13.84	12.72	13.06	4.79	4.55	4.82	....
Average.......	51.86	49.12	49.16	13.84	12.67	13.09	4.79	4.53	4.77	....
W. L. Whitehouse, Moosic, Pa.	51.52	51.27	51.47	13.40	13.42	13.57	4.77	4.73	4.78	4.76
	51.55	51.27	51.43	13.38	13.42	13.54	4.77	4.80	4.73	4.72
	51.52	....	....	13.42	13.42	....	4.77	4.74	4.78	4.76
Average.......	51.53	51.27	51.45	13.40	13.42	13.55	4.77	4.76	4.76	4.74
General average.....	51.57	50.31	51.03	13.41	12.91	13.11	4.66	4.55	5.27	4.71

[1] Omitted from average.

The following results were reported by Mr. B. Apel, Dr. Roemer, Dr. Hansen, and Dr. Kamman, official chemists of the Chamber of Commerce, Berlin, using the platinic and the perchlorate methods for samples 1 and 2 and the perchlorate method for No. 3. "The analyses were made exactly according to the methods prescribed for the laboratories of the potash works. Only the averages are here reported. The various findings agreed well with one another and did not deviate more than 0.1 per cent." Averages: Sample No. 1, 51.45 per cent of potash; sample No. 2, 13.27 per cent; sample No. 3, 4.99 per cent.

These determinations are very interesting as showing the agreement between the German and American methods.

COMMENTS BY ANALYSTS.

H. H. Hill, Blacksburg, Va.: I would suggest that a large porcelain dish be substituted for the Erlenmeyer flask in the cobalt volumetric method. The erlenmeyer when hot is difficult to handle and the precipitate of manganese hydroxid which sticks to the sides is somewhat hard to dissolve, whereas with the open dish it goes into solution easier, the agitation of the solution being better accomplished by the use of a long stirring rod. In this way the oxidation of the potash precipitate is more thorough, the unoxidized particles showing up against the white background. The procedure is also shortened. I would also suggest the use of a porous clay crucible instead of the gooch, as it eliminates any error that may creep in from the use of asbestos. These crucibles may be purchased from any of the leading dealers in chemical apparatus.

J. S. Burd, Berkeley, Cal.: I merely desire to say that it appears to me that the results obtainable by the cobalti-nitrite method vary so greatly with slight changes in the manipulation that I can not conceive that it will prove to be a method which can be used to advantage in an ordinary analytical laboratory. It would seem that the factors causing these variations are of such a nature that they could only be controlled in a research laboratory by elaborate precautions. As at present developed it could not be used in a laboratory where it is necessary to turn out large quantities of work. Again I do not see that there is any great saving either in the matter of cost of reagents or time in the gravimetric method and little, if any, in the volumetric determination, as compared with the platinic chlorid method. The method does not seem to be sufficiently promising from any point of view to justify further work on the part of the association.

O. M. Shedd, Lexington, Ky.: I did not obtain very good results last year on the chlorid and kainit, but excellent results on the mixed fertilizer. The work this year shows the same thing and the only reason I can give for it is the larger amount of potassium present in samples Nos. 1 and 2. I never have thought that this method would always prove reliable when very large amounts of potassium are to be precipitated, due mainly, I believe, to the reagent used not being stable. There are some inconsistencies in my results which I have not been able to account for as yet, for instance, the results obtained on weighing the potassium salt and afterwards titrating the same with permanganate. Along this line I think cooperative work should be done, for it will avoid the error of two separate determinations and at the same time show whether the precipitate has a constant composition. My results seem to indicate that the composition is not constant.

Arao Itano, East Lansing, Mich.: The volumetric cobalti-nitrite method gives lower results than either the official method or gravimetric cobalti-nitrite method. The gravimetric cobalti-nitrite method gives slightly lower results than the official method, except with sample No. 3, in which case higher results were obtained in every instance where the method as outlined was followed. The failure to get results agreeing with the official method on samples 1 and 2 may be explained by the small aliquot portions used.

Cornelius Beatty, College Park, Md.: The gravimetric cobalti-nitrite method gives results which agree with those given by the official method. It takes more time than the official method. Two hours are required to evaporate the excess of the cobalti-nitrite reagent to the required sirupy consistency, but the platinic-acid solution used in the official method can be evaporated in three-quarters of an hour. A part of the precipitate is apt to cling to the porcelain dish. This increases the time necessary to remove it. The precipitate attains constant weight after drying 54 minutes, but if it is weighed after drying 30 minutes the error in weighing a half gram precipitate is less than five ten-thousandths of a gram. Perhaps this method might be modified so that it would be as short as the official method. The principal merit of the method lies in the circumstance that platinum is not used as a reagent, which is important in consideration of the present price of platinum and the work of recovering it.

The results obtained by the volumetric cobalti-nitrite method were not satisfactory. In order to remove the precipitate from the porcelain Gooch crucible, it is best to dry it. If the process of heating the precipitate with the potassium-permanganate solution is performed in a beaker, the porcelain crucible may be immersed in the potassium-permanganate solution with the adhering precipitate and removed after the determination is complete. The precipitate dissolves slowly in the potassium-permanganate solution. The purple color of the potassium-permanganate solution disappears after one hour. The oxalic acid dissolves the manganese dioxid very slowly. The solution does not become perfectly colorless, but retains a faint pink which is not changed by further additions of oxalic acid.

The gravimetric cobalti-nitrite method is identical with the volumetric cobalti-nitrite method as far as the process of obtaining the precipitate is concerned. After the precipitates are obtained it is easier and quicker to weigh them than to treat them as described in the directions for the volumetric method. The volumetric cobalti-nitrite method has no advantage over the gravimetric cobalti-nitrite method.

L. T. Bowser, Dayton, Ohio: The gravimetric method gives results varying about half as much as the volumetric, the difference between extremes by the latter giving about 2 or 3 per cent per 100 per cent of potash (K_2O), and I note that others quite uniformly secure about the same differences. The acid phosphate and potash give results that are about 30 per cent high on the gravimetric method, which strikes me as peculiar. Taken as a whole, from the standpoint of a chemist, considering the method for the first time (as nearly as I can imagine it), the procedure is not inviting from the point of view of simplicity. There are entirely too many ifs and ands to consider, too many conditions calling for predetermination of the nature of the sample to be examined. All these may be kept in mind easily enough by those of us who have spent several years at trials of the method, but the young analyst or the older one who has always secured good results by the platinum method is apt to indefinitely postpone the use of the cobalti-nitrite procedure.

J. H. Roop, La Fayette, Ind.: In the determination of potash by the gravimetric method, in drying the final precipitates obtained from samples No. 1 and No. 2, two hours were required before constant weight was obtained. In the case of samples No. 1 and No. 3 there is apparently a tendency toward too high results when potash is determined by the gravimetric method, due probably to the presence of some impurities which are not carried away by wash water.

In the case of the gravimetric method on sample No. 3, 50 cc of the solution were measured into a platinum dish and sulphuric acid (1:1) was added before the ammonia was driven off. The results obtained were 7.43 per cent and 7.22 per cent. This part of the method was modified by taking aliquots, evaporating to dryness, and then adding the dilute sulphuric acid. The results obtained are those reported in the table. The presence of ammonia probably caused higher results in the former instances. There seems to be no saving in time by the use of either the gravimetric or the volumetric method as compared with the official method and accuracy is undoubtedly sacrificed for cheapness of reagents.

G. F. Lipscomb, Clemson College, S. C.: The method as outlined in the directions sent out for analysis was first carefully tried, but was found to give such discordant results, uniformly from 3 to 5 per cent lower than the official method (Chemistry Bulletin 107, Revised), that the following modification was made in the directions received: Instead of evaporating the solution and precipitate to a pasty consistency over a boiling water bath, we evaporated the potash solution to about 5 cc on a boiling water bath, then removed the flame and added the precipitating agent, after which the evaporation to a pasty condition was continued with the bath just below the boiling point. The precipitate was bright yellow in color. The precipitate obtained in the manner directed by the referee was always dark colored.

Thos. C. Pinkerton, Philadelphia, Pa.: In addition to the three methods specified, I titrated the precipitate weighed in the gravimetric cobalti-nitrite method. Potassium permanganate was standardized against sodium oxalate, tested as described in Treadwell (translated by Hall, vol. 2, p. 555). The cobalti-nitrite determinations were carried almost to dryness and were a thin paste when cold. The loss of potassium by this method seemed to be in proportion to the amount in the sample, No. 1 showing the greatest loss and No. 3 practically none. I can not account for the high results in No. 3 with the gravimetric method.

F. B. Porter, Atlanta, Ga.: From our limited experience with these methods I should say that they are unsatisfactory on potash salts.

Paul Rudnick, Chicago, Ill.: We find that the results by the volumetric cobalti-nitrite method agree fairly well with those obtained by the official chlor-platinate method, but we get consistently high results by the gravimetric cobalti-nitrite method. This is particularly so in the case of sample No. 3. In this case there is a serious lack of agreement both between the two analysts and among their individual results. I regret that this sample was exhausted before we could locate the source of the trouble. It seems very probable, however, that the presence of acid phosphate is a factor in the difficulty.

We also find that the proposed modified official method gives higher results than the original method; and inasmuch as this proposed modification differs in no essential manner from that in use on phosphoric acid for similar purposes, it seems to me that the association should adopt this modification in justice to the fertilizer manufacturers.

In comparing the official chlor-platinate method with the cobalti-nitrite method as outlined for this work, we are very favorably impressed with the great improvement in the character of the precipitate as a result of evaporating to a sirupy consistency only, instead of to dryness as formerly. The precipitated cobalti-nitrite behaves nearly as well as the chlor-platinate precipitate, except for the fact that it does not settle as quickly.

P. F. Trowbridge, Columbia, Mo.: We are all of us disappointed in the cobalti-nitrite method. There is no question but that by continued repetition greater accuracy could be obtained by either the volumetric or gravimetric methods. I feel that a method to become official should be one that a good chemist can secure concordant results by paying careful attention to the descriptions, even if he is not making these determinations continuously. The gravimetric method is no gain in time over the official method and does not seem to me worthy of further consideration. The volumetric method has good possibilities when the details are a little more definitely settled.

ADDITIONAL WORK REPORTED BY COLLABORATING ANALYSTS.

O. M. Shedd: The following determinations were made by first weighing the potassium salt obtained by the cobalti-nitrite method and afterwards titrating the same with fifth-normal potassium permanganate. In drying the salt all were constant in one hour.

Percentage potash determinations—Modification 1.

Method.	No. 1.	No. 2.	No. 3.
Gravimetric cobalti-nitrite method	52.18 52.40	13.48 13.51	4.48 4.45
Average	52.29	13.50	4.47
Volumetric cobalti-nitrite method (on same precipitate as above)	50.69 51.03	13.05 13.07	4.24 4.32
Average	50.86	13.06	4.28

I also made some determinations by evaporating the solution to 5 to 10 cc before adding the nitrite reagent.

Percentage potash determinations—Modification 2.

Method.	No. 1.	No. 2.
Volumetric cobalti-nitrite method	[1] 50.80 [2] 50.34 [3] 50.17	[1] 13.63 [3] 12.99 [4] 13.18
Average	50.44	13.07

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[1] 0.20 gram and 20 cc reagent; 0.50-gram and 15 cc reagent.
[2] 0.10 gram and 15 cc reagent; tenth-normal potassium permanganate and tenth-normal oxalic acid used.
[3] 0.50 gram and 20 cc reagent; tenth-normal potassium permanganate and tenth-normal oxalic acid used.
[4] 0.25 gram and 15 cc reagent; tenth-normal potassium permanganate and tenth-normal oxalic acid used.

The results on Nos. 1 and 2, while low, will average higher when the solution is evaporated to 5 to 10 cc before adding the reagent. On No. 3, on which better results were obtained, this was recommended in the directions. I have a sample of solid sodium cobalti-nitrite that is being sold for these determinations, and it seems to me that if it proves satisfactory it will be of great assistance, as it will do away with the unstable cobalti-nitrite solution.

Thomas J. Pinkerton reported the following results obtained by titrating the gravimetric precipitate:

Comparison of gravimetric method and of titrating the gravimetric precipitate.

Gravimetric.	Volumetric and gravimetric.	Gravimetric.	Volumetric and gravimetric.	Gravimetric.	Volumetric and gravimetric.
49.39	49.47	12.07	12.29	5.25	4.50
49.18	48.53	12.12	12.22	5.20	4.62
49.30	48.79	12.37	12.39	5.44	4.57
				5.38	4.48
49.29	48.93	12.19	12.30	5.32	4.54

R. M. Pinckney also titrated the gravimetric cobalti-nitrite precipitate after weighing. He says: "I am unable to explain why this titration did not agree with those which were titrated before drying." His results are as follows:

Titration of gravimetric precipitate after weighing.

Sample 1.	Sample 2.	Sample 3.
46.7376	10.5459	Lost.
47.6792	10.5802	4.5710
48.1072	10.5117	4.5368
47.8504	....	....

McCreary and Hibbard report the following additional work:

P. L. Hibbard: Preliminary tests indicated that correct results were not usually obtained, accordingly the following work was done in the hope of finding some modification that would give good results. The method of handling the precipitate after it is obtained seems satisfactory, except that there appears to be no good reason for diluting the permanganate so much; 100 cc of water makes it easier to handle. The results are much influenced by the length of time of evaporation and the amount of dilution of reagent or volume evaporated. The reagent may be entirely decomposed by heating with water a few hours. Referee's instructions were followed closely except in regard to the volume of water added at the beginning of the evaporation. Blank tests indicated so little that no correction was made for them on either gravimetric or volumetric methods. The filter tubes with precipitates for the gravimetric determination were dried at 100 cc for three hours. A little loss was noted during the second hour, but none in the third hour. Results are given in the order in which obtained, the work extending over about 10 days. If the time of evaporation could be accurately controlled, and if the proper dilution of each sample could be known without previous experiment, it appears probable that accurate results would be obtained; but lacking these essentials, the method must, in my opinion, remain unreliable.

Results on sample No. 1, aliquot used=0.2 gram.

Volume.[1]	Time.[2]	Potash (K_2O).	Method and remarks.	
cc.	*Hrs.*	*Per cent.*		
20	2½	48.85	Both run at same time.	
20	2½	48.42		
45	2½	48.93	Volumetric cobalti.	All run at same time to determine possibility of securing uniform results by uniform conditions using the same solution.
45	2½	48.93		
45	2½	49.01		
45	2½	48.50		
45	2½	48.93		
45	2½	49.27		
45	2½	48.20	Gravimetric cobalti.	
45	2½	49.80		
	Min.			
20	60	50.70	Volumetric cobalti.	
20	60	50.15		
5	50	50.00	....do..	All run at same time and same solution.
5	30	50.07		
5	30	50.46	Gravimetric cobalti, very difficult to filter.	
5	30	50.35		
5	30	51.06	Volumetric cobalti—all run at same time and same solution.	
5	30	51.30		
5	34	51.14		
5	42	50.80		
5	46	51.22		
	Hrs.			
35	2	50.15	Volumetric cobalti—all run at same time, following referee's directions as nearly as possible.	
35	2	49.43		
35	2	48.66		
35	2	50.54		

[1] Volume=volume of potash solution when reagent was added.
[2] Time=length of time heated in evaporating dish on steam bath.

Previous work showed that more nearly correct results were obtained by decreasing volume and time. The last set was run to see if increased skill and familiarity with the method obtained by practice would enable us to get good results by following the referee's instructions exactly.

Results on sample No. 2, aliquot = 0.5 gram.

Volume.[1]	Time.[2]	Potash (K_2O).	Method and remarks.	
cc.	*Hrs.*	*Per cent.*		
25	2	12.40	Volumetric.	All run at same time.
25	2	12.57		
25	2	12.70		
25	2	12.95	Gravimetric.	
25	2	13.00		
	Min.			
5	35	13.16	Gravimetric.	A little precipitate ran through filter and was lost.
5	35	....		Lost, could not filter.
5	35	13.06	Volumetric; last four all run at once.	
5	35	13.18		

[1] Volume=volume of potash solution when reagent was added.
[2] Time=length of time heated in evaporating dish on steam bath.

Results on sample No. 3, aliquot=1 gram.

Volume.[1]	Time.[2]	Potash (K_2O).	Method and remarks.
cc.	*Hrs.*	*Per cent.*	
20	2	4.50	Volumetric. All run at same time.
20	2	4.49	
20	2	4.47	
20	2	4.47	
20	2	4.84	Gravimetric.
20	2	4.77	
	Min.		
5	30	5.32	Gravimetric. All run at same time.
5	30	5.39	
5	30	4.51	Volumetric.
5	30	4.53	
5	30	4.64	Volumetric—run at same time.
5	30	4.56	
5	30	4.51	

[1] Volume=volume of potash solution when reagent was added.
[2] Time=length of time heated in evaporating dish on steam bath.

P. L. McCreary: A number of determinations made on chemically pure potassium sulphate by the volumetric cobalti-nitrite method, adhering closely to the referee's directions, gave low and erratic results and in a general way indicated that the length of time of evaporation and the dilution were important factors to be considered. The following determinations were, therefore, made, varying the volume of the potash solution before addition of the cobalti-nitrite reagent and also the time of evaporation.

Results by the volumetric cobalti-nitrite method, varying volume of solution and time of evaporation.

Sample and volume of solution.	Time.	Potash (K_2O).	Remarks
cc.	*Min.*	*Per cent.*	
Sample 1—Muriate of potash:			
20	90	52.25	Firm on cooling.
20	90	52.25	
20	70	52.25	Thick sirup on cooling.
20	70	52.25	
20	60	49.20	Filtrate pink, indicating decomposition of reagent.
20	60	49.60	
20	60	49.30	
20	50	51.23	Thin sirup on cooling.
20	50	51.48	
5	35	51.64	Firm on cooling.
5	35	51.64	
5	35	50.90	
Sample 2—Kainit:			
5	30	13.24	
5	30	13.34	
5	30	13.27	

At the conclusion of all experimental work the following set of determinations was made according to the referee's directions in order to see whether, after more experience with the method, better results might not be obtained.

Final results on sample No. 1.

Volume.	Time.	Potash (K_2O).
cc.		*Per cent.*
35	1 hour 50 minutes	50.20
35	1 hour 50 minutes	50.46
35	1 hour 50 minutes	50.63
35	2 hours	51.48

The results seem to indicate that more nearly accurate and uniform results are obtained using a small volume of potash solution and limiting the time of evaporation. The reagent is decomposed by continued heating, and this decomposition is hastened by dilution. It is necessary to have quite a large excess of the undecomposed reagent present at the conclusion of the evaporation to secure good results. Using small volumes of the potash solution, thus shortening the time of evaporation, tends to produce the desired effect. For any given set of determinations run at the same time under identical conditions of dilution and rapidity of evaporation, fairly concordant, if incorrect, results may be obtained. Certain standard conditions might be found which, if closely followed, would give correct results. It would be, however, very difficult if not impossible to duplicate exactly the necessary conditions from time to time, and as the slightest variations produce erratic results the method in its present imperfect state is not reliable.

Arao Itano reported a modification of the gravimetric cobalti-nitrite method. His comments and results are as follows:

In seeking for some explanation of the high results given by the gravimetric cobalti-nitrite method on sample No. 3, it was found that the precipitate contained some phosphate. It was, therefore, believed that if all the phosphates were removed from the solution before treating with the cobalti-nitrite reagent the results might agree with those obtained by the other methods. At this point all of sample No. 3 had been used, although a quantity of solution was still on hand. This solution had been obtained in the usual way by boiling 10 grams for one-half hour with 300 cc of water. By testing this solution it was found that phosphate was present. Four portions of 50 cc each were placed in beakers and numbered. Nos. 1 and 2 were treated with a small quantity of milk of lime, allowed to stand a few minutes, filtered, washed with water, and the excess of lime in the filtrate removed by precipitating with ammonium oxalate in the usual manner. After filtering and washing with water the filtrate was evaporated to dryness and from this point the method was followed exactly as given. Nos. 3 and 4 were treated with baryta water, filtered, washed with hot water, and excess of baryta removed with sulphuric acid, the filtrate evaporated to dryness, ignited, and from this the method was followed as given.

The following table gives the results obtained as compared with those by the official and volumetric cobalti-nitrite methods:

Potash results on sample No. 3 by three methods.

Official method.	Volumetric cobalti-nitrite method.	Gravimetric cobalti-nitrite method—phosphates removed.
Per cent.	*Per cent.*	*Per cent.*
4.67	4.41	4.47 (1)
4.47	4.47	4.38 (2)
4.69	4.37	4.31 (3)
....	4.30	4.31 (4)
....	4.38	....
....	4.44	....

The removal of the soluble phosphates in this way, however, is rather tedious, as it requires the filtering and washing of two precipitates. Consequently the following method was adopted:

Weigh out samples and boil with water as usual. Then make slightly ammoniacal, add 5 cc of milk of lime, and allow to stand on hot plate for one hour; cool, make up to volume, and filter. A sample of commercial fertilizer collected in the open market was used for further work.

Potash results on a commercial fertilizer by three methods.

Official method.	Gravimetric cobalti-nitrite method untreated.	Gravimetric cobalti-nitrite method treated.
Per cent.	*Per cent.*	*Per cent.*
10.28	11.75	10.49
10.39	11.61	10.49
	12.04	10.41
	12.06	10.42

H. H. Hill, associate referee, has called attention to the fact that the factors for calculating potash from K_2PtCl_6 as found in Bulletin 107, Revised, Bureau of Chemistry, vary somewhat from those calculated according to the latest atomic weights. I have recalculated these factors according to the International atomic weights for 1912 (J. Amer. Chem. Soc., 1911, 33:11), and find they should be as follows:

Potassium platinic chlorid to potassium chlorid, 0.3067; to potassium sulphate, 0.3585; to potassium oxid, 0.1938.

For the sake of uniformity the revised factors should be adopted.

Discussion of Methods.

I. VOLUMETRIC COBALTI-NITRITE.

This method has now been studied by the association for three successive years, and though it may appear from the many variable results that we are still far from having a method which will give satisfaction in the hands of the average worker, much progress has been made, many interesting points have developed, and certain questions fundamental to its accuracy have been answered.

A careful examination of the data for each of the past three years shows very close agreement for the mixed fertilizers; but in the case of potash salts there is a decided tendency to low results. This is probably due in part to the inability to precipitate completely the large amount of potash in solution. Several analysts have, however, consistently obtained excellent agreements for all the samples tested, showing that with experience it is possible to obtain concordant results.

In justice to the method I wish to say that I believe for the determination of small amounts of potash in such materials as soils, cereals, etc., it can be used with great accuracy, and for fertilizers and substances high in potash, success will depend largely upon the analyst, practice being an important factor. In its present stage of development the method is rather complicated, requiring many operations and too close attention to minor details to prove attractive for control work. As many of the results have been variable and the comments so generally unfavorable, it does not seem best to continue work on this method until it has been further developed. Investigations are now in progress in several laboratories and it is probable that radical changes will be made which will tend toward greater accuracy.

II. GRAVIMETRIC COBALTI-NITRITE METHOD.

Although this method has also proved more or less variable, it is simpler than the volumetric and requires much less time to complete a series of determinations. Very high results were generally obtained upon sample No. 3, owing probably to the interference of phosphates, as this sample was composed of acid phosphate and muriate.

Mr. Arao Itano, of East Lansing, Mich., has reported a modification of this method by which he obtained very accurate results. Briefly, his procedure is to throw out the phosphates with milk of lime before precipitating with the cobalti-nitrite reagent. With this improvement the gravimetric method is so promising that I think it deserves further study by the association.

III. MODIFIED OFFICIAL METHOD.

It is well known that the official method of making up the solution does not recover all of the soluble potash present in a mixed fertilizer. Recent investigations have shown that this is probably due to the formation of insoluble potash compounds when samples containing iron, alumina, and phosphates are boiled with water. Several different ways of getting this potash in solution have been tried from time to time, but for various reasons, mainly owing to the use of acid for extraction, they have not met with the approval of the association. The proposed modification of washing a weighed amount of sample upon filter paper with boiling water has been tried for the past two years with very satisfactory results.

In the work of 1910[1] an increase of potash recovered of from 0.1 to 0.3 per cent was quite generally reported; the average for the official method being 4.69 per cent, that for the modified method 4.81 per cent, a difference of 0.12 per cent in favor of the latter. About the same increase has been obtained this year, the averages being 4.66 per cent official and 4.71 per cent modified official—a difference of 0.05 per cent in favor of the modification (theory, 4.85).

Last year[1] the referee made a comparison of the results on 32 different samples by both methods, which resulted very favorably for the proposed modification. Twenty-seven samples gave increased amounts of potash ranging from 0.05 to 0.54 per cent. In one sample there was no difference. Four samples gave slightly less. It often happens that both methods agree, but in the majority of cases the modification gives the highest results; the increase, especially for high-grade goods, being sometimes as great as 0.8 per cent to 0.9 per cent.

The method is more accurate than the official, as it recovers more of the soluble potash and gives results closer to the theory; as it also complies with the laws of those States requiring water-soluble determinations, it seems to the referee that it should be adopted as official.

RECOMMENDATIONS.

It is recommended—

(1) That work on the volumetric cobalti-nitrite method be discontinued until further investigation has simplified it and rendered it of more practical value.

(2) That a further study of the gravimetric cobalti-nitrite method be made by the next referee, in the case of mixed fertilizers, using the modification suggested by Mr. Itano of precipitating the phosphates with milk of lime before adding the cobalti-nitrite reagent.

(3) That the proposed modification of the official method be adopted, and that the official method, Bulletin 107, Revised, page 11, (2) "Methods of Making Solution," be revised to read as follows:

(*a*) *With potash salts and mixed fertilizers.*—Weigh 2.5 grams of the sample upon a 12.5 cm filter paper and wash with successive small portions of boiling water into a 250 cc graduated flask to a volume of about 200 cc. In the case of mixed fertilizers add 2 cc of concentrated hydrochloric acid, heat to boiling, and add to the hot solution a slight excess of ammonium hydroxid and then sufficient ammonium oxalate to precipitate all the lime present; cool, dilute to 250 cc, mix, and pass through a dry filter.

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[1] U. S. Dept. Agr., Bureau of Chemistry Bul. 137, p. 16.

In the case of muriate and sulphate of potash, sulphate of potash and magnesia, and kainit, dissolve and dilute to 250 cc without the addition of ammonium hydroxid and ammonium oxalate.

Under (3) "Determination," second line should be changed to read 0.5 gram instead of 1 gram.

On page 12—

(*b*) *Muriate of potash.*—Acidify 50 cc of the solution prepared according to (2) (a) with a few drops of hydrochloric acid, etc.

(*c*) *Sulphate of potash, sulphate of potash* and *magnesia*, and *kainit.*—Acidify 50 cc of the solution prepared according to (2) (*a*) with a few drops of hydrochloric acid, etc.

(4) That the factors for calculating potash from potassium platinic chlorid (Bul. 107, Rev., Bureau of Chemistry, p. 12) be revised to read as follows:

(c) FACTORS.

For the conversion of potassium platinic chlorid to potassium chlorid use the factor 0.3067; to potassium sulphate, 0.3585; to potassium oxid, 0.1938.

ESTIMATION OF POTASSIUM AS THE COBALTI-NITRITE.

By W. A. DRUSHEL.

In 1907 and 1908 the writer published three papers[1] on a modification of the Adie and Wood volumetric method for potassium and the application of the modified procedure to fertilizers with less than 10 per cent of potassium oxid (K_2O) and to soils and physiological products. It was shown that with proper care the Adie and Wood gravimetric cobalti-nitrite method under the newly described conditions of precipitation and the modified procedure for the volumetric method for small amounts of potassium both give results fairly accurate and concordant with the results obtained by the platinic chlorid method. In no case, however, in the results obtained and published was more than 0.1 gram of potassium oxid used for a determination. The potassium factor, 0.000856 or 0.000857, for strictly tenth-normal potassium permanganate was derived for the modified volumetric procedure from experimental data and theoretical considerations published in the first paper.

The application of the volumetric cobalti-nitrite process to the estimation of potash in soils and such physiological products as urine, blood, lymph, serum, and milk is fully described in papers just cited.

That the modified volumetric procedure may be applied with a fair degree of accuracy for potassium in small amounts (though in somewhat larger amounts than "1 to 50 parts per million"), contrary to a recently published statement,[2] is shown by the writer's published results, and has been further verified by the results of V. C. Myers,[3] of New York, along physiological lines, and by the results of the referees on soils published in Bulletins 132 and 137 of the Bureau of Chemistry.

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[1] Amer. J. Sci., 1907, 24: 433; ibid., 1908, 26: 329 and 555; reprinted in Zts. anorg. Chem., 1908.

[2] Bowser, J. Amer. Chem. Soc., 1911, 33: 1752.

[3] Privately communicated.

Synopsis of referees' reports on total potassium in soils for 1909 and 1910.

Year and number of sample.	Number of determinations made.		General averages in per cent.	
	J. L. Smith gravimetric method.	Volumetric cobalti-nitrite method.	J. L. Smith gravimetric method.	Volumetric cobalti-nitrite method.
1909: [1]				
No. 1	11	11	1.508	1.531
No. 2	36	26	1.661	1.663
1910: [2]				
No. 1	13	11	1.854	1.863
No. 2	13	10	[3] 1.739	1.642
No. 3	12	9	1.956	1.951

[1] U. S. Dept. Agr., Bureau of Chemistry Bul. 132, p. 25.
[2] U. S. Dept. Agr., Bureau of Chemistry Bul. 137.
[3] An erroneous computation reported in Bulletin 137 should read 1.670.

In the report of the association for 1909 it was requested by the referee on potash that the determination be made on sample 1 (potassium chlorid of tested purity) and on sample 2 (a complete mixed fertilizer containing a considerable proportion of organic matter) by the official method and also by the proposed volumetric cobalti-nitrite method. The volumetric method was outlined for the cooperating analysts by the referee, it being recommended that not more than 0.2 gram of sample 1 be used; that 1 gram of sample 2 and 10 cc of the cobalti-nitrite reagent be used for each determination; or that for sample 1 an amount of solution corresponding to not more than 0.2 gram of the sample be used. Nine cooperating analysts reported results, some of which were obtained by introducing slight modifications to the method as outlined. These results have been examined to determine the extreme variations by the two methods and the comparative numbers of determinations reported lying within fairly wide limits of variation.

Extreme variation of results by the official and volumetric cobalti-nitrite methods in 1909.

Method.	Sample 1.	Sample 2.
	Per cent.	*Per cent.*
Official	62.35 to 63.21	3.13 to 3.93
Volumetric	61.77 to 63.44	3.30 to 3.90
	60.07 to [1] 64.44	

[1] Obtained by a modification of the prescribed procedure.

Proportion of results lying within the limits specified.

Method.	Between 62.5 and 63 per cent.	Between 3.6 and 3.85 per cent.
Official	12 out of 15	10 out of 19.
Volumetric	7 out of 31 [1]	14 out of 28.

[1] 10 of these results lying below 62.50 per cent were reported by the same analyst.

A tendency toward low results was frequently observed in the volumetric method, particularly in sample 1. This may be due to insufficient use of sodium cobalti-nitrite in making the precipitation, since this reagent on heating is partially decomposed by the acetic acid which is present. The writer in developing the method did not consider 20 to 25 cc of the precipitating reagent to be more than a liberal excess for 0.2 gram of potassium (K_2O) when used in the presence of acetic acid, instead of 10 cc, as recommended by the referee. The presence of acetic acid appears to be necessary to produce a filterable precipitate, as was shown by Adie and Wood.

In 1910 it was recommended that potash in samples 1 (commercial muriate), 2 (kainit), and 3 (a complete mixed fertilizer) be determined by the official and the volumetric cobalti-nitrite methods, etc. For the volumetric and gravimetric cobalti-nitrite methods it was suggested that 5 cc of the solution of sample 1, equal to 0.1 gram, be run from a burette into a porcelain evaporating dish, diluted to 20 cc, 1 cc of glacial acetic acid and 10 cc of sodium cobalti-nitrite solution be added, etc.

From the results of 17 cooperating analysts the following table of extreme variations, etc., has been obtained:

Extreme variation of results by official and volumetric cobalti-nitrite methods in 1910.

Method.	Sample 1.	Sample 2.	Sample 3.
	Per cent.	*Per cent.*	*Per cent.*
Official	50.6 to 53.26	12.28 to 13.73	4.39 to 4.99
Volumetric	[1] 49.55 to 55.26	[1] 12.11 to 13.35	[1] 4.26 to 4.91
Reported general average by official method.	51.77	12.73	4.69

[1] One analyst reported considerably lower on samples 1 and 2, 46.1 per cent and 10.27 per cent; one analyst reported 4 per cent for sample 3.

Proportion of results lying within the limits specified.

Method.	Between 51.5 and 52 per cent.	Between 12.5 and 13 per cent.	Between 4.6 and 4.75 per cent.
Official	23 out of 42.	26 out of 36.	22 out of 37.
Volumetric	13 out of 49 [1]	27 out of 49 [2]	17 out of 52.[3]

[1] Five of these results, all below 51.5 per cent, were reported by the same analyst.
[2] Seven results below 12.5 per cent reported by same analyst.
[3] Eleven results, all below 4.6 per cent, reported by two analysts.

Under "Discussion of results" in Bulletin 137, page 21 (1910), the referee says: "Owing to the use of such a small aliquot the variations in sample No. 1 appear to be greater than the actual error occurring in the determination. In calculation this error is increased ten times. The use of a larger aliquot should be studied." In the conclusions the referee further says: "By the volumetric method, as outlined, the majority of analysts have obtained satisfactory results. * * * Although it is difficult to draw definite conclusions from the data received, from my experience with the method I think that with slight modification an accurate optional method can be developed."

From the writer's experience in developing and applying the volumetric method and from his published results it would appear that it was never intended that the method should be applied in practice to cases where the error in the determination will be increased 10 times in the calculation. It must be admitted that the method has certain inherent difficulties leading to errors, which, however, by proper care in manipulation and proper restriction in the application of the method may be kept within fairly reasonable limits, as has been amply shown by the soil analysts and physiological chemists. Obviously a method intended for substances running under 10 per cent in potassium (K_2O) when tested on such high-grade potash salts as the muriate should be given the advantage of every care and precaution which would make for accuracy. When a 50 per cent potash salt is so aliquoted that only 5 cc may be used for a determination it requires unusual care in measuring this amount from a burette to avoid the introduction of an appreciable error at this point. An error of 1/40 cc (not an unusual error for analysts accustomed to gravimetric rather than volumetric work) in the two burette readings necessary in measuring out 5 cc of solution may lead to an error of 0.5 per cent in the final result $(2\times1/40 \text{ cc})\div5 \text{ cc}\times50 \text{ per cent}=0.5$ per cent. Is it fair to test a method intended for small amounts of potash on a 50 per cent salt and recommend to the cooperating analysts such a small amount of solution of the salt to be used that in measuring from a burette this amount (5 cc) there is the possibility of introducing in the final result an error of 0.5 per cent due to burette manipulation alone? Would it not have been preferable to have the solution sufficiently dilute so that 25 cc instead of 5 cc subsequently diluted to 25 cc with water would have been measured from the burette?

In the matter of concordance in results reference may be made to the work of Shedd (Bulletin 137, pp. 20–22), who has had considerable experience with the volumetric method, both in soils and in fertilizers.

In the use of the cobalti-nitrite precipitating reagent in the presence of acetic acid care must be exercised to keep an ample excess of reagent present during the evaporation, since the reagent is decomposed by acetic acid at the temperature of the steam bath. At least 15 to 20 cc of the reagent should be used for 0.1 gram of potassium oxid if the precipitation and evaporation are made in the presence of 1 cc of added glacial acetic acid. The results for 1910 on commercial muriate generally run low, and if for 1911 the aliquots for the volumetric process have been made on the basis of 5 cc of the potash solution to 0.1 gram of potassium (K_2O), and not more than 10 cc of the precipitating reagent used in the presence of variable amounts of glacial acetic acid, equally low results may be expected from the use of this method on a 50 per cent potash salt.

In conclusion the writer would suggest that the volumetric method be not rejected by reason of results obtained on a 50 per cent potash salt under conditions not very favorable in the hands of analysts unfamiliar with the method, but rather that it be limited to low-grade potash substances, for which it was originally developed and for which satisfactory results have been obtained by a number of analysts. With a more liberal use of the precipitating reagent and more favorable conditions of manipulation in the hands of analysts familiar with the limitations of the method, reasonably uniform and dependable results should be obtained from the use of the volumetric method in fertilizers containing less than 10 per cent of potassium oxid.

The following paper was presented by the referee on potash, Mr. Baker, at the request of the author.

SOME FEATURES OF THE COBALTI-NITRITE METHOD FOR POTASSIUM.

By Leon T. Bowser.

The writer has been interested in this method from the first, and in addition to his published work has at hand an accumulation of data which it is purposed to put at the service of the association and its members, believing that an early solution of the cause of difficulty is more probable with numerous investigators studying it than with only one.

The procedure falls naturally into two divisions, precipitation and titration. The method of precipitation is one first published by W. A. Drushel, and practically no change has thus far been made in it. The titration method is an evolution from that proposed by Drushel, but is quite different from that advocated by the writer. The latter at one time stated, as nearly everyone has seemed to believe, that the titration is defective, but that the precipitation is fairly satisfactory. As will be shown, however, exactly the opposite is the case, and all research should be directed toward the discovery of a satisfactory method of precipitation. The chief causes of difficulty in the present one have been worked out, and will be first discussed, followed by a description of a reliable titration procedure, and lastly will be detailed by a description to work out a satisfactory new precipitation method,

DIFFICULTIES IN PRECIPITATION.

Many have found that dilution of the potash solution, volume of the reagent, presence or absence of acetic acid, time, temperature, and degree of evaporation, and various other conditions produce unexpected changes in the final condition of the precipitate, and with some combinations results are high, with others low. Discussion of the variations due to these minor conditions is useless, however, since there are two major ones entirely overshadowing them in importance. One is the effect of certain metallic salts during evaporation, the other the solubility of the yellow precipitate in solutions of cobalt salts, and in a contrary direction the precipitation under certain conditions of little-known bluish-green cobalt compounds.

Salts of such ordinary metals as iron, aluminum, manganese, barium, calcium, magnesium, and copper, as well as many of the less common ones, are those apt to give trouble. Di-potassium-sodium cobalti-nitrite when heated nearly to boiling (as happens during evaporation) in solutions of such salts, is more or less completely decomposed, leaving a pink solution. With some the reaction is very slow, with others almost instantaneous, but there is a remarkable lack of uniformity about the behavior of the tests carried out as exact duplicates. So serious is this difficulty that under certain conditions it would mean failure to attempt the precipitation of potassium in their presence. Sometimes a fairly accurate result may be attained even in the presence of such salts, but frequently the writer has had duplicate determinations give results, one nearly correct, the other but a fraction of the amount of potash present. This is a serious obstacle to the use of the method, and it apparently can not be avoided when using the evaporation procedure, save by removing all metals except sodium and potassium.

To the fact that the precipitate is more or less soluble in solutions of cobalt salts may be attributed the balance of our difficulties. Shedd, in his second paper,[1] approached, but seemingly did not discover, this feature of the subject. He states that the precipitate is acted on by the acetic acid and water present, but concludes that a large excess of reagent is necessary to keep all the potash in solution, while in reality the action of acetic acid and water at boiling is entirely overshadowed by the solvent action of the cobalt salts resulting from decomposition of the reagent. So far as his tests go, they accord fully with the writer's own observations, but to the fact that the reagent is decomposed by heating to boiling, leaving, it is probable, a mixture of primary salts, is due the principal trouble.

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[1] J. Ind. Eng. Chem., 1910, 2: 379.

Upon adding the reagent to a potash solution in the cold, the yellow triple nitrite quickly precipitates and settles. No sooner does the supernatant solution become thoroughly warmed than it begins to lose its characteristic wine color and assumes a reddish tint changing to purple. As the amount of solution grows less the precipitate disappears, and in many cases there is a stage where but a few grains remain to view. Then as the concentration increases there is a redeposition of the nitrite, and by the time pastiness is reached nearly all is precipitated again, although concealed by the purplish cobalt salts.

Titrations of the precipitate at different stages of evaporation confirm fully the fact that at certain times a large part is actually dissolved, and that later it is again deposited. In this case, also, there is a remarkable lack of uniformity; sometimes the action is very complete, at others hardly noticeable. In numerous qualitative tests with various salts of cobalt as well as with the reagent, it was found that sometimes part and sometimes all of the triple nitrite goes into solution upon boiling, while upon cooling there are cases where a considerable part is redeposited, and others where never a trace of the precipitate reappears. With some of the latter a small amount was thrown down, however, by adding alcohol, hence the precipitate is somewhat soluble in cold solutions of cobalt salts.

In the light of these facts part of the irregularities of volumetric results are easily explained. During evaporation some of the precipitate goes into solution, but the degree of solution is not uniform, being influenced by factors too numerous and elusive to control. Evaporation to pastiness, which has been found desirable, leaves variable amounts of cobalt solution, accompanied by a proportionately varying amount of the precipitate, which is subsequently washed away in filtering. This must always produce a loss of potash.

In the opposite direction, under certain imperfectly understood conditions, there is deposited along with the yellow precipitate a bluish-green substance insoluble in water, which subsequently takes up permanganate just as does the precipitate. Shedd has shown that the amount of this substance is variable, and at times equivalent to a very considerable amount of potash. This is the positive source of error, and it depends on whether conditions favor the formation of this bluish-green substance or the retaining of part of the precipitate in the pasty cobalt solution as to whether we have high or low results. It is not improbable that the securing of a result nearly correct is due largely to the balancing of these two sources of error.

TITRATION PROCEDURE.

It has been found, notwithstanding all statements to the contrary, that strictly accurate results are attainable by using as little water as possible, adding permanganate, introducing 5 cc of sulphuric acid (1:1), and then heating just to boiling (stirring constantly meanwhile), decolorizing with oxalic acid, and titrating back with permanganate. Perfect decomposition of the precipitate is always secured, and by keeping the solution well stirred there is no loss of nitrous acid. The time necessary for a complete titration should not exceed three or four minutes.

Since the method of precipitation is faulty, proof of the entire accuracy of this titration procedure could not be shown by titration of precipitations from potash solutions, but the writer has been able to establish it in another way. This reaction and precipitate seem especially designed for the detection and estimation of cobalt, and in a paper to appear later the writer will present a qualitative test so sensitive that one part of cobalt in twenty million of water can be detected. A perfect precipitation of cobalt is easily attained, and its amount is capable of estimation by means of permanganate; hence, with this source of error entirely eliminated, if there occurred any noticeable deviations the trouble would manifestly lie in the method of titration. A large number of successive titrations has shown that the deviation is exactly the same as that between successive titrations of oxalic acid by permanganate; hence no error is introduced by the use of a small volume of water and the addition of sulphuric acid before heating.

ATTEMPTED IMPROVEMENT OF PRECIPITATION.

It must be confessed at the outset that up to the present the effort to secure a satisfactory substitute for the evaporation procedure has not been altogether successful, but some lines of attack have proved encouraging, and ultimate success appears not improbable. The writer has previously shown that when the reagent is added to a potash solution and the precipitation allowed to stand overnight without heating the amount of potash recovered varies with the time of standing and the concentration of the original solution. The introduction of ethyl alcohol, in addition to throwing down a precipitate too finely divided to allow of proper washing, gives unreliable results. Heating such a solution is entirely inadmissible, as the precipitate is partially decomposed in this way.

The substitution of acid-alcohol (one part 95 per cent alcohol, one part glacial acetic acid) for alcohol as an accelerator of precipitation, as recently described by the writer, gives a better texture of precipitate, but the results are no improvement over those obtained by the evaporation procedure. A quantity of pure, dry sodium cobalti-nitrite was obtained, and a number of trials was given it. A sufficient amount was dissolved in water to make a reagent equivalent to that prepared in the regular way, and acetic acid was added in like amount. As thus made up the reagent gave results, both by the evaporation and the acid-alcohol procedures, that were extremely irregular, although almost invariably too low. Later, however, with a different formula for the reagent, results of considerable promise were secured, which will be described.

From the action of boiling solutions of cobalt salts on the yellow precipitate it appeared that an investigation of their behavior in the cold would be desirable, and qualitative studies were made which gave results both helpful and convincing. The most favorable condition for throwing down a perfect precipitate is when there is a large excess of potassium salts. Nearly although not altogether as desirable is a well defined excess of sodium nitrite, while the presence of a large excess of cobalt is a very decided detriment. The formation of sodium cobalti-nitrite demands very little formality; whenever sodium nitrite is added to a solution of a cobalt salt and acetic acid this reagent is formed, no matter what the ratio of cobalt to sodium nitrite, and there is no necessity for adding enough cobalt to combine with all the sodium nitrite used. When the amount of cobalt is but slightly in excess of that required for complete precipitation of the potash a much better precipitate is secured than when it is in large excess. In fact, with certain excesses of cobalt no yellow precipitate is obtained, but a small amount of a gray one, while with a greater excess precipitation does not occur at all. The conclusion can not be escaped that heretofore a grave error has been made by having too much cobalt present during precipitations.

There are several points of importance regarding manipulation which it would be well to have in mind before discussing the behavior of reagents of various kinds. In all cases except one (to be described later) it has been found best to place the potash solution in a beaker, most conveniently of 250 cc size, and evaporate until only enough solution remains to moisten the bottom thoroughly. After cooling, and before the reagent is added, the solution is whirled around so as to moisten every place where dried salts are visible. Precipitating in a beaker has the advantage, for volumetric work, that the asbestos and precipitate are thrown back into it, and it is unnecessary to remove what adheres to the walls.

It has seemed the best practice to add the reagent very slowly, with constant agitation of the solution in the beaker. Decidedly higher results are secured if the reagent is added suddenly and agitation carried out afterward. If acid-alcohol or any other accelerator is added it should be allowed to wash down the sides of the beaker, accompanied by frequent agitations. It is well to allow a precipitation to stand about five minutes before adding an accelerator. After a precipitate has been filtered it should be titrated as soon as possible, since a long delay produces a loss of potash. The entire method possesses the merit that it can be carried out with ease and accuracy in artificial light.

In harmony with conclusions from the qualitative tests previously described, several reagents were now prepared which contained but a small amount of cobalt, computations showing that about one-tenth as much as usually employed should be sufficient. Of the many prepared, only two (numbered 5 and 7 of the series) were finally deemed promising enough to justify further work. In addition to those that failed, mention should be made of cobalt nitrite, with which it was hoped to obtain the precipitate tri-potassium cobalti-nitrite, but which gave very poor results. In the preparation of these reagents the potassium present as an impurity in the sodium nitrite precipitates out, and must be filtered off. It should be noted that to a greater or less degree all cobalt salts, especially if in contact with acetic acid, are affected by light, and hence should be kept in the dark. The fact must also be borne in mind that asbestos, no matter how carefully purified nor how free from organic matter, takes up a definite amount of permanganate, which must be allowed for. The same asbestos may be used over and over, but it does not thereby lose its affinity for permanganate.

Reagent No. 5 is prepared as follows: Dissolve 1.9 grams of dry sodium cobalti-nitrite and 19.8 grams of sodium nitrite in water, allow to stand for one hour, filter to remove the precipitate which settles, and make up to 100 cc. By observing the precautions regarding manipulation previously described a number of procedures involving this reagent have given very fair, although not entirely uniform results. These procedures are: (1) Slow addition of the reagent to a potash solution; (2) addition after introducing 1 cc of glacial acetic acid; (3) addition of the reagent followed by 10 cc acid-alcohol; and (4) addition preceded by 1 cc of acetic acid and followed by 10 cc of acid-alcohol. Standing for some time, usually from fifteen minutes to two hours, is required to give a complete precipitation. Precipitates secured in these ways are in general of excellent texture, and give no trouble in filtering and washing.

Some very promising results were also obtained by evaporating the potash solution down to about 5 cc and adding 10 cc of acid-acetone (one part acetic acid, one part acetone), followed as usual by 10 cc of reagent No. 5. An extremely good precipitation results, and filtering and washing is very rapid, but results are usually a little high. Very little time could be devoted to this procedure, but it is worthy of further attention.

Reagent No. 7 is prepared as follows: Dissolve 1.1 grams of cobalt nitrite and 19.8 grams of sodium nitrite in water, allow to stand for some time, filter off the muddy brown precipitate, and make to 100 cc. Some good results were obtained with this reagent by the use of procedures 2, 3, and 4 above. Further attention is justified in this case also, as there are many very good points about the reagent.

Lack of time has prevented exhaustive tests of the many possibilities offered by these reagents, but it seems probable that some of the procedures, or methods evolved from them, will ultimately prove successful. More detailed information regarding any of the points in this paper will be gladly furnished by the writer, and it is hoped that in it may be found some facts that may prove of aid to those interested in the development of the cobalti-nitrite method for potassium.

REPORT ON SOILS.

By J. G. LIPMAN, *Referee*, and G. S. FRAPS, *Associate Referee.*

The referee on soils for 1911 was instructed: (1) To investigate the subject of a more accurate method for humus determinations. (2) To make further studies of the modified cobalti-nitrite method. (3) To continue the study of methods for the quantitative estimation of soil acidity.

In accordance with these instructions the work on humus determinations was undertaken by the associate referee, and the results secured are included in this report.

The work on methods for estimating soil acidity could not be carried out in any satisfactory manner because of the lack of exact and reliable working methods. The referee on soils was led, therefore, to test out certain bacteriological soil-acidity work. Since investigation has not been carried sufficiently far to draw final conclusions, a report of progress is herewith presented, and it is hoped that additional data will be secured for the consideration of this association.

I. BACTERIOLOGICAL METHODS FOR THE ESTIMATION OF SOIL ACIDITY.

By J. G. LIPMAN.

As the culture medium for a host of bacteria and of other microorganisms the soil must possess a suitable reaction. If this condition is met the desirable changes will proceed normally. The organic matter will undergo rapid decomposition, ammonia and nitrates will be produced, and carbon dioxid will be evolved in sufficient amounts to provide for the transformation of relatively inert mineral compounds into available plant food. Moreover, a suitable reaction will make possible the fixation of nitrogen by symbiotic and nonsymbiotic bacteria (azo-bacteria), and the economical utilization of legumes for increasing the store of combined nitrogen in the soil. On the other hand, increasing acidity will retard more and more the fermentative changes in the soil, and the bacteria themselves will dwindle in numbers. Finally the soil may become unfit for the natural growth of higher and lower plants and will cease to yield profitable harvests.

Recognizing these facts in an empirical way, farmers in many lands have adopted the practice of applying various kinds of lime and marl at more or less regular intervals. Such applications serve to neutralize the organic acids that tend to accumulate in cultivated land. In this manner the reaction of the soil is not allowed to become too acid for the activities of the desirable bacteria. However, the farmer is often at a loss to know the exact or even approximate amount of lime required for his land. Excessive applications are not desirable, since they needlessly increase the cost of crop production and may lead to too rapid dissipation of the store of soil nitrogen.

Hence the farmer finds himself obliged to invoke the aid of the soil chemist in determining the lime requirements of his land. Unfortunately, however, the chemist is not in a position to supply the desired information. The methods at his disposal for estimating soil acidity are crude and unreliable, as shown by the experience of members of this association. It is for this reason that the referee on soils was instructed to make further study of promising methods. In searching for methods that might prove more accurate than those already tested, it occurred to him to utilize the soil bacteria themselves as indicators of soil reaction. Since such common organisms as *B. mycoides*, *B. subtilis*, Azotobacter, etc., are sensitive to changes in the reaction of the medium, it was not difficult to devise a bacteriological method for the quantitative estimation of soil acidity. The basis for a method of this sort may be found in the following facts:

(1) Organisms like *B. mycoides* fail to produce characteristic growth in nutrient bouillon when the medium has an acidity greater than 2 per cent.

(2) Additions of acid soil to neutral or slightly acid bouillon may increase the acidity of the medium so as to prevent the characteristic development of the organisms.

(3) By adjusting the quantity of soil necessary to modify the reaction of the medium the acidity of the soil itself may be determined.

In accordance with these facts 10 cc portions of nutrient bouillon were placed in test tubes together with varying quantities of soil corresponding to 0.5 gram, 1 gram, 2 grams, 5 grams, and 10 grams. The tubes were then plugged, sterilized, cooled, and inoculated with *B. mycoides*. It was found that the smaller quantities of soil exercised a depressing effect, and that the larger quantities of soil entirely stopped the growth of the bacteria. A considerable number of soil samples was thus tested, and fairly concordant results were secured. It soon became evident, however, that the depressing effect of the soil might have been due to factors other than acidity. This belief was strengthened by the common knowledge that soil sterilized by heat may for a time become unfit for bacterial growth. Hence a quantity of soil was sterilized by being kept in contact with 95 per cent alcohol for some hours. It was then gently heated to drive off the excess of alcohol and placed in test tubes containing sterilized bouillon. The contents of the test tubes were inoculated with *B. mycoides*. Similar quantities of alcohol-sterilized soil were placed in test tubes containing a solution of mannite and mineral salts and inoculated with Azotobacter. Characteristic growths occurred in most of the tubes, and the depressing effects of soil acidity became particularly apparent in the mannite-soil cultures inoculated with Azotobacter.

Since this is only a report of progress, no attempt will be made to quote figures. It should be added here, however, that the toxic substances produced in sterilizing soil are partly or wholly neutralized by addition of calcium carbonate prior to sterilization. Hence the proposed method may be modified in that the quantity of the bouillon and soil used may be kept constant. On the other hand, variable amounts of calcium carbonates may be added and the effect on the bacteria noted. The data thus far secured seem to indicate that a simple and accurate method could be devised, perhaps, in accordance with the facts noted. For this reason it seems desirable to continue the work in accordance with the suggestions here outlined.

II. REPORT ON HUMUS.

By G. S. Fraps.

At the request of Dr. Lipman, referee on soils, the writer consented to undertake the work on humus and prepare a report on it. The work of the year was confined to a study of the methods for preparing a solution as nearly as possible free from soil particles. This appears to me to be at present the most important part of the problem. The method of preparing the humus solution is also of importance and should be subjected to study.

The following instructions were sent out to those who had expressed a willingness to cooperate:

DIRECTIONS FOR HUMUS WORK, 1911.

Samples.—Three samples of soil are sent. The sample should be mixed thoroughly before any portion is taken for analysis.

Work outlined.—At present the most important point in the humus estimation is the elimination of the clay. We have, therefore, selected two methods for removing the clay for test. The method of solution is not official, but should give concordant results and is used for convenience.

Preparation of solution.—Digest 20 grams of the soil for five hours with 400 cc of fifth-normal hydrochloric acid. Filter on a fluted filter and wash thoroughly. Cover and allow to drain overnight. Measure out 1,000 cc of 4 per cent ammonium hydroxid (1 cc=11.55 cc fifth-normal hydrochloric acid), and wash the soil into a glass-stoppered bottle, digest for twenty-four hours with shaking every hour during the working day, and let settle twenty-four hours. Decant about 800 cc through a folded filter into a dry glass-stoppered bottle and use aliquots of the solution for analysis. Shake the bottle well before withdrawing portions for analysis.

(*a*) *Official method.*—Evaporate 100 cc to dryness in a platinum dish, dry for four hours in a boiling-water oven, and weight. Ignite and weigh again. Report loss on ignition and ash.

(*b*) *Mooers-Hampton method.*—Evaporate 100 cc to dryness in a porcelain dish and heat for two hours on a water bath. Take up with five or more successive portions of about 20 cc each of 4 per cent ammonium hydroxid and decant into another dish. Evaporate again, heat as before, take up with ammonium hydroxid, filter into a platinum dish, and wash. Evaporate the filtrate to dryness and complete as in method (*a*).

(*c*) *Rather method.*—Place 130 cc in a glass-stoppered cylinder and add 0.65 gram of ammonium carbonate. Allow to stand overnight, filter through a dry filter into a dry flask, and measure out 100 cc for evaporation in the platinum dish. Complete as in the official method.

Remarks.—Please express your opinion in regard to the three methods and give us the benefit of any other observations concerning the matter. An early report will be appreciated.

G. S. FRAPS, *Associate Referee,*
College Station, Tex.

RESULTS OF THE WORK.

The results on humus are reported in Table 1 and those on humus ash in Table 2.

TABLE 1.—*Percentage of humus in soils.*

Analyst.	Sample No. 1.			Sample No. 2.			Sample No. 3.		
	Official.	Mooers and Hampton.	Rather.	Official.	Mooers and Hampton.	Rather.	Official.	Mooers and Hampton.	Rather.
W. T. McGeorge, Honolulu, Hawaii	5.02	0.75	0.87	0.30	0.25	0.27	0.46	0.30	0.25
	5.39	.83	.86	.34	.27	.28	.51	.32	.24
N. C. Hamner, Texas	5.47	.67	.67	.33	.32	.32	.33	.28	.28
J. B. Rather, Texas	5.00	.65	.66	.24	.29	.22	.24	.20	.26
K. W. White, Pennsylvania	.93	.75	.80	.22	.19	.27	.22	.17	.27
	.84	.74	.81	.25	.21	.31	.22	.16	.28
G. W. Walker, Minnesota	3.26	.74	.61	.28	.29	.14	.32	.32	.17
W. B. Ellett, Virginia	5.43	....	.80	.45	....	.24	.31	.00	.40
	5.03	....	....	.42	....	....	.35	....	.00
C. S. Robinson, Michigan	4.40	.47	.78	.20	.25	.23	.26	.26	.26
	4.57	.55	1.02	.21	.27	.22	.26	.27	.25
	4.73	.59	1.01	....	....	....	.24	.27	.24
	4.73	.50	.71	....	....	....	.24	.26	.26
H. C. McLean, New Jersey	5.52	.77	.71	.30	.39	.22	.37	.30	.27
	5.49	.75	.76	.27	.36	.23	.37	.33	.26
	5.02	.75	....	.26	.25	....	.38	.29	....
	5.11	.71	....	.27	.25	....	.35	.24	....
	....	....	....	....	.24	....	....	....	....
	....	....	....	....	.24	....	....	....	....
	....	....	....	....	.28	....	....	....	....
E. Van Alstine, Illinois	5.03	1.07	.67	.24	.42	.19	.34	.40	.26
	5.24	.95	.73	.16	.50	.14	.39	.46	.23

TABLE 2.—*Percentage of humus "ash" in soils.*

Analyst.	Sample No. 1.			Sample No. 2.			Sample No. 3.		
	Official.	Mooers and Hampton.	Rather.	Official.	Mooers and Hampton.	Rather.	Official.	Mooers and Hampton.	Rather.
W. T. McGeorge, Honolulu, Hawaii	34.00	0.17	0.14	0.38	0.30	0.41	1.15	0.22	0.20
	34.59	.26	.14	.47	.32	.50	.50	.37	.28
N. C. Hamner, Texas	37.01	.18	.47	.29	.36	.18	1.45	.33	.38
J. B. Rather, Texas	33.00	.33	.24	.25	.49	.22	1.22	.40	.22
J. W. White, Pennsylvania	.68	.33	.27	.33	.13	.29	.46	.13	.41
	.68	.32	.30	.34	.12	.29	.46	.13	.42
G. W. Walker, Minnesota	24.88	.79	.16	.32	.30	.34	1.20	.55	.23
W. B. Ellett, Virginia	39.09	....	.28	.45	....	.47	1.90	....	.50
	38.93	....	....	.42	....	....	1.88	....	.00
C. S. Robinson, Michigan	28.95	.45	.51	.40	.39	.29	1.36	.41	.47
	28.83	.48	1.00	.39	.41	.28	1.36	.44	.44
	29.03	.48	1.96	....	....	....	1.32	.45	.50
	29.11	.44	.32	....	....	....	1.33	.45	.53
H. C. McLean, New Jersey	33.40	.48	.09	.18	.00	.24	1.40	.40	.27
	33.18	.53	.14	.20	.04	.26	1.31	.30	.23
	33.81	.31	....	.33	.07	....	1.53	.33	....
	34.61	.38	....	.33	.14	....	1.48	.34	....
	....	....	....	....	.13	....	....	....	....
	....	....	....	....	.14	....	....	....	....
	....	....	....	....	.20	....	....	....	....
E. Van Alstine, Illinois	36.27	1.47	.44	.37	.73	.27	2.22	1.12	.46
	35.59	1.16	.27	.34	.72	.30	2.11	.98	.51

REMARKS OF ANALYSTS.

W. P. Kelly, Honolulu: The determinations reported were made by Mr. William T. McGeorge, of the department, who comments on the methods as follows: "Either the Mooers-Hampton or the Rather method gave satisfactory results, both as regards humus and humus ash. The Rather method can be worked in a little less time than that of Mooers-Hampton. but either is satisfactory for such soils as these. The official method is entirely unreliable with heavy clay soils." I may add that neither the Mooers-Hampton nor the Rather method, when applied to such soils as ours, effects a complete removal of the clay.

N. C. Hamner, Texas: The Rather method I consider best, as it is quickest and gives a very low ash, while the Mooers-Hampton has to be evaporated at least twice and the residue extracted several times; even then, in my judgment, it loses a part of the humus originally extracted from the soil occluded by the clay. The agreement between the Mooers-Hampton and Rather methods is very satisfactory, but certainly in No. 4671 the lime was not all extracted, nor was all organic matter.

J. B. Rather, Texas: All lime was not removed from sample No. 2 by the acid. Fresh or tested ammonia solutions should be required.

William Frear, Pennsylvania: The results obtained by the first three methods reported represent as exact a following of the details of the procedure indicated by you as was possible. Mr. White notes that in No. 2 the quantity of carbonate of lime was so great that it neutralized most of the acid used for the removal of calcium and magnesia from their humic combination prior to the alkaline treatment. Supplementary to the results by your methods are duplicates by the use of the Chamberland tube method. This gives results more closely approaching those of the Mooers-Hampton method than do the results secured by the other two methods named, so far as the ash constituents are concerned, although in the case of samples Nos. 2 and 3 the ash in the Chamberland filtrate is higher than the Mooers-Hampton method yielded. The same is true of the organic portion of the filtrate in these two cases. [In reply to a subsequent letter, Dr. Frear wrote:]

I note your comment on the low ash report by Mr. White. Thereby hangs a detail. Mr. White has been working on allied questions for two or three years and has endeavored to diminish the ash by awaiting patiently the subsidence of the suspended mineral matter prior to filtration. Other factors in the results seem not to have been materially changed by this practice, but the elimination of clay as a source of ignition and loss has been largely accomplished. I do not believe, however, that this method would solve the difficulty in case of certain soils, such as the Cuban soils, whose behavior led me a decade ago to the suggestion for the need of improved water-soluble alkaline-humus method to eliminate the error due to the water of hydration contained in the mineral materials taken into the filtrate.

Results by the Chamberland tube method.

Sample.	Humus.	Ash.
	Per cent.	*Per cent.*
No. 1	0.67 .70	0.26 .26
No. 2	.22 .20	.23 .24
No. 3	.25 .24	.16 .17

G. W. Walker, Minnesota: In making the determinations for humus I have carefully followed your directions. In regard to the preparation of the solution, it would seem to be preferable to weigh out sample of soil on filter and leach with hydrochloric-acid solution until the filtrate gives no test for lime, as I find that unavoidably clay passes through the filter in filtering and washing as you direct. Also in using a fluted filter there is some difficulty in washing free from soil on transferring to flask for treatment with ammonia solution.

As to the three methods for making the determination, I am very well pleased with the Rather method. It appeared to give very good results as far as the removal of clay is concerned. It occurs to me, however, that possibly some of the humus or albuminous matter in the humus might be precipitated by the ammonium carbonate along with the clay. On the whole, it appears to me that the Rather method is an excellent modification of the official method, and, if adopted, would no doubt lead to more accurately comparable results.

Along with the results obtained by the methods you have given, I have tabulated the figures obtained by running the solution in a centrifuge at the speed of approximately 1,500 revolutions per minute.

Results obtained with the centrifuge.

Sample.	Humus.	Humus ash.
	Per cent.	*Per cent.*
No. 1	2.91	18.47
No. 2	.23	.43
No. 3	.24	.65

C. S. Robinson, Michigan: It was found that after standing 24 hours there was still a large amount of clay suspended in the solution to be used for analysis. The effect of this is shown in the amount of ash in samples Nos. 1 and 3 by the official method, which uses this solution directly for the determination. In both cases the amount of ash obtained in this method was much higher than in either of the other methods. In sample No. 1, which gave the largest amount of suspended matter, the humus results are much higher than the results with the other two methods. This is due, in all probability, to the driving off of the moisture from the ash. On the other hand, while the quantity of ash obtained by the official method in sample No. 3 is approximately three times as great as that obtained by either of the other methods, the average amount of humus is less than that obtained by the Mooers-Hampton and just equal to that obtained by the Rather method. This may, of course, be accounted for by the assumption that the water-holding capacities of the two kinds of ash differed. In sample No. 2 the supernatant liquid was fairly clear, and the effect of the ash was not so marked.

As to the relative merits of the three methods, both the Mooers-Hampton and Rather methods seem to be preferable to the official method. Between the first two there is but little choice though the Rather method requires a little less time on the part of the operator.

The writer is of the opinion that a considerable error may have been introduced into all of this work by the method of preparation of the solution. After filtering the soil from the acid solution it was found quite difficult to remove it from the filter paper without either contaminating it with fibers of the paper or leaving some soil on the paper. It would seem better to follow the official method in this respect or to follow one similar to that which has been in use in this laboratory for some time in the volumetric determination of phosphoric acid. By the latter method the soil would be filtered from the acid solution through a pad of ignited asbestos formed on a perforated porcelain plate placed in a common glass funnel. When the soil has been thoroughly washed the porcelain plate, asbestos, and sample can be easily transferred to a wide-mouthed, glass-stoppered bottle for digestion with alkali. This prevents any possibility either of losing any of the sample or of adding organic matter to it from the filter paper.

H. C. McLean, New Brunswick, N. J.: The first results, in each case, were obtained before becoming accustomed to the methods. The Rather method seems to me to be the best, as it is short and eliminates the clay better than the Mooers-Hampton method. The results seem to correspond very well with the Mooers-Hampton method also.

J. H. Pettit, Urbana, Ill.: Mr. E. Van Alstine, of this station, has done this work. He has no particular comment to make upon the relative value of the methods, and I may say that the results go far to confirm us in the idea that the determination of humus in soils is extremely unsatisfactory. We feel that so far as our work is concerned the determination of one definite constituent of all organic matter, as, for instance, carbon, is a much better indication of what the soil contains. We, of course, realize that the percentage of carbon varies in organic compounds, and that the relative amounts of these various organic compounds are not always present even in the same soil.

CONCLUSIONS AND RECOMMENDATIONS.

The present official method for humus is very inaccurate on some soils, on account of the clay carried in suspension. Both the Mooers-Hampton and the Rather methods are much more nearly correct. The Rather method is more rapid than the Mooers-Hampton. The centrifuge method, as reported by Mr. Walker, does not remove the ash. The subsidence method, tested by Mr. White, is slow, and I am inclined to think with Mr. Frear that it will not be effective on some soils. It is possible that in the Chamberland tube method, tested by Mr. White, some humus does not pass through the tube.

The associate referee recommends further study of the Rather method with a view to its adoption as an official method by this association. The present official method should be abandoned, as it does not remove all the clay.

The referee then presented the following paper on behalf of the author:

DETERMINATION OF CARBON DIOXID IN SOILS.

By LEON T. BOWSER.

In his 1909 report[1] on carbonates in soils the associate referee tentatively suggested that a volumetric method for the purpose would be desirable and might ultimately be found. The present one, at that time in an advanced stage of development, has since been perfected to the point where it is believed it can be used by nearly any analyst. It is based on one described by J. C. Mims,[2] and the procedure is essentially that of releasing carbon dioxid by means of hydrochloric acid, absorbing it in a strong alkaline solution, and measuring its amount by titration with a standard acid.

Absorption is accomplished in a tower especially designed to meet the conditions. No preliminary guard tubes are necessary, and instead of rigidly excluding water from contact with the potash solution it is the practice to distill over a small amount, thus insuring the mechanical carrying over of residual carbon dioxid along with the water vapors. Estimation of the amount of carbon dioxid absorbed is accomplished, as before stated, by titration with a standard acid.

Previous to titration the absorbent solution contains a mixture of potassium hydroxid and carbonate. In titrating with an acid, using phenolphthalein as indicator, disappearance of the pink color marks the point at which all hydroxid has been neutralized and the normal carbonate has been converted to bicarbonate, or, in other words, the normal carbonate has been half neutralized:

$$KOH+HCl=KCl+H_2O$$
$$K_2CO_3+HCl=KHCO_3+KCl$$

The titration being continued, after adding a drop of methyl orange, appearance of the usual acid reaction denotes complete neutralization of the bicarbonate:

$$KHCO_3+HCl=KCl+CO_2+H_2O$$

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[1] U. S. Dept. Agr., Bureau of Chemistry Bul. 132, p. 30.

[2] Ibid., Bul. 65, p. 156.

The volume of acid used in this latter titration, then, is just one-half of that required to release all the carbon dioxid from the condition of a normal carbonate. It follows that 1 cc of normal acid used in the titration between the two end points is equivalent to 0.044 gram of carbon dioxid.

The form of apparatus used is shown in figure 2; F is a flask in which the carbonate is decomposed by an acid, which is introduced through a small separatory funnel, S, or the substitute shown in figure 2, b. The condenser, C, is so constructed that the inner tube is quite short and of small bore. All tubing used in the apparatus, except the body of the tower, is of 2 mm internal diameter, which allows very little space for carbon dioxid to collect and possibly escape absorption. Complete details of the construction and dimensions of the absorbing tower, T, are given in the figure.

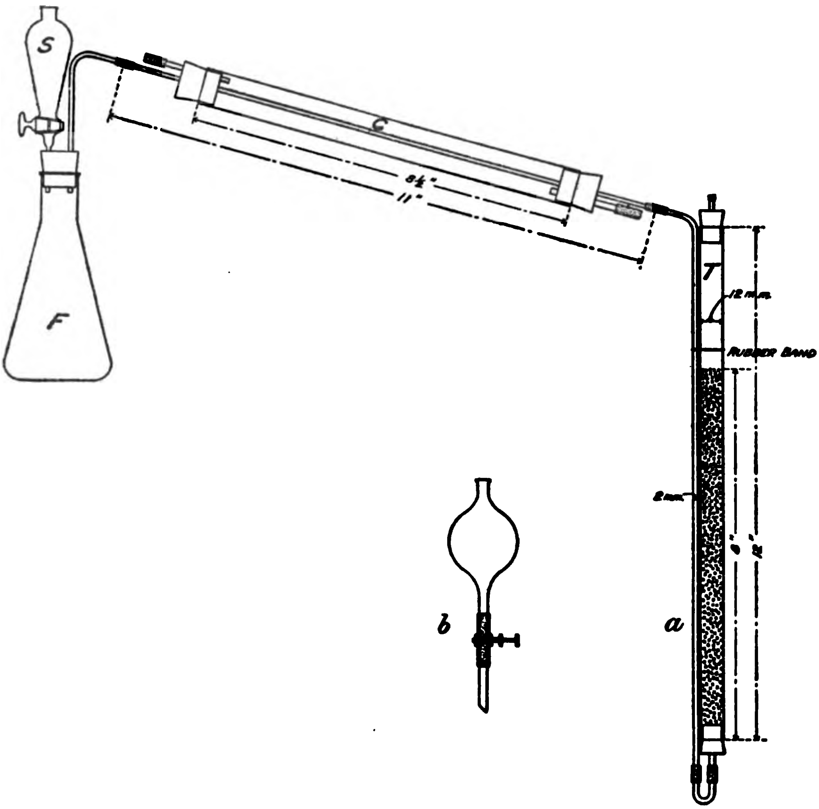

FIG. 2.—Apparatus for determination of carbon dioxid in soils.

A suitable amount of soil, usually about 10 grams, is placed in a 100-cc Erlenmeyer flask, then about 50 cc of water is added. The apparatus is connected up, 10 cc of absorbent solution (50 grams of potassium hydroxid in 100 cc solution) pipetted into the tower, and sufficient hydrochloric acid introduced through S to decompose all the carbonates in the flask. With very effervescent soils the acid is let in a few drops at a time; with others the entire amount is added at once. After active effervescence has ceased the flask is heated, taking care not to force bubbles through the tower too fast, and water is allowed to distil over until the tower is nearly filled.

The carbonated solution is then transferred to a 100-cc volumetric flask, the tower thoroughly rinsed into the flask, and the contents of the latter made up to volume. A 25-cc aliquot is pipetted out and placed in a 250-cc beaker. A few drops of phenolphthalein are added, followed by 10 to 15 cc of 95 per cent alcohol, then acid of approximately normal strength is run in until the color begins to dim, after which decinormal acid is used to complete the discharge of the pink color. The burette reading is recorded, a drop of methyl orange added, and the titration continued to the usual end point. The latter burette reading is set down, and after subtracting the equivalent of carbon dioxid in the reagents the difference in cubic centimeters between the two, multiplied by the factor 0.0044 for strictly decinormal acid, gives the grams of carbon dioxid in the aliquot, from which the percentage in the original sample may be easily found. The amount of carbon dioxid in the reagents is ascertained by a blank determination carried out in the same way as the regular one.

Soils containing 0.1 per cent or less of carbon dioxid require for titration an acid of a strength not exceeding fiftieth-normal, since when stronger ones are used the carbon dioxid is equivalent to but a fraction of 1 cc and too great an error is thus introduced. An occasion may arise where a centinormal acid is demanded, although the writer has never encountered such a case. Even with fiftieth-normal acid much care is necessary in observing end points, and the use of comparison solutions is advisable.

It is probable that the titration will be found the most difficult point in the use of the method. Ordinarily the phenolphthalein end point is supposed to give no trouble, but it is very apt to do so nevertheless. The writer has discovered that the addition of a small volume of ethyl alcohol, after introducing phenolphthalein, totally eliminates all such difficulties, but without this precaution it is improbable that a satisfactory end point can be secured. During this part of the titration the solution should be kept rotating vigorously, since any local excess of acid would carry the reaction on to the point of releasing carbon dioxid from the bicarbonates in the immediate vicinity.

In the addition of methyl orange not more than one drop should be added to 25 cc of the carbonated solution. When this amount is used the change of color is quite easily noted, but the presence of larger quantities renders the observation a matter of difficulty. The end point to observe is when the clear lemon yellow of the alkaline solution becomes a shade darker from the admixture of the pink of the acid reaction just beginning to take place. When the acid used for titration is no weaker than decinormal the color change is plainly noticeable, but with acids more dilute it is advisable to have at hand a comparison solution of the same tint as the titrated solution when alkaline, and the slightest difference of color is then easily noted in a good light. Even thus, a considerable amount of care must be exercised should an acid as dilute as hundredth-normal be in use, but even this end point is distinguished with accuracy.

The very best of results were obtained on chemically pure carbonates by this method, but for the present paper only the results on soils will be given. As a preliminary experiment two determinations were made on a sort of artificial soil containing a known amount of carbon dioxid. A very pure sea sand was ignited for some time to remove all organic matter and decompose such calcium carbonate as might be present. This was cooled and to a weighed portion was added enough analyzed calcium carbonate to give exactly 0.05 per cent of carbon dioxid. A blank determination was first made on the water, reagents, and freshly ignited sand, then two determinations were carried out on the artificial soil. The blank first obtained was deducted from the titration results, and the carbon dioxid was calculated from the difference. The determinations gave 0.043 and 0.048 per cent carbon dioxid, an average of 0.0455 as against the 0.05 per cent actually present, which seemed very satisfactory.

There was available a quantity of each of the two samples sent out for the 1909 association soil work, and advantage was taken of the fact to compare this method with the gravimetric one. Four determinations were made on each of these soils, under conditions parallel to those used by the association workers, and the results are given in the table, accompanied by a comparison with the figures obtained by the gravimetric method. The averages given in the association report are: Soil 1, 0.081 per cent; soil 2, 0.027 per cent carbon dioxid, but these include the results of only five analysts, two others reporting too late to be considered. For the present comparison all of the results are taken into account, giving the averages as shown.

Comparison of averages by the volumetric and the association (gravimetric) methods.

Method.	Soil 1.	Soil 2.
Volumetric method	0.080	0.026
	.077	.031
	.075	.031
	.084	.026
Average for volumetric method	.079	.028
Average for association (gravimetric) method	.078	.028

The agreement between the revised association averages and the volumetric method is very close, and shows that the latter is thoroughly reliable. On the score of consistent results the volumetric method is decidedly superior to the gravimetric, the variations between duplicates being only about one-third as great.

Equally favorable results have been secured on routine samples, and no difficulty has ever been experienced in getting good duplicates. The writer believes that a thorough trial of the method by the association will show that it is very accurate, that the results are uniformly concordant, the manipulation is simple, and the apparatus easily constructed and not in the least fragile. These advantages, coupled with the fact that there are very few precautions to be observed, should make it of great service in soil analysis. A more detailed description of the method and the results secured by it will appear a little later in one of the periodicals.

NOTE ON POT EXPERIMENT SHOWING GAINS IN NITROGEN WHEN CERTAIN LEGUMES WERE GROWN.

By B. L. Hartwell and F. R. Pember.

The following table shows the net gain in nitrogen per pot 12 inches in diameter during a five-year pot experiment in which, without nitrogenous manuring, the legumes mentioned below were grown each summer and harvested; whereas, in the winter, vetch was grown in the greenhouse in all the pots and mixed with the soil at blossoming time:

Name of crop.	Pot number.	I. Nitrogen in soil at the beginning. (The variation is due to different proportions of pebbles.)	II. Nitrogen in soil at beginning and in added seeds and water, not including that in vetch.	Oven-dry crops removed in five years.	Average per cent of nitrogen in the same.	III. Nitrogen in the aerial portion of the summer legumes removed.	Per cent of nitrogen in dry fine soil at end (0.1502 per cent at the beginning).	IV. Nitrogen in soil at end of the five-year period.	V. Nitrogen in soil at end and in crops removed (IV + III).	Net gain in nitrogen during the experiment (V−II).
		Grams.	*Grams.*	*Grams.*	*P. ct.*	*Grams.*	*P. ct.*	*Grams.*	*Grams.*	*Grams.*
Soy bean	[1] 1	30.20	35.68	346	3.10	10.73	0.1994	40.10	50.83	15.15
	[2] 2	29.43	34.91	351	3.07	10.76	.1992	39.03	49.79	14.88
	3	29.43	34.91	362	3.10	11.22	.1995	39.08	50.30	15.39
	4	30.01	35.50	357	3.10	11.07	.1967	39.30	50.37	14.87
Adzuki bean	5	29.33	32.23	190	2.58	4.91	.1887	36.85	41.76	[3] 9.53
	[2] 6	29.89	32.79	203	2.24	4.55	.1913	38.07	42.62	[3] 9.83
	7	27.61	30.58	314	2.08	6.54	.1906	35.04	41.58	11.00
	[1] 8	29.58	32.54	296	2.28	6.76	.1961	38.62	45.38	12.84
Cowpea	13	28.45	31.22	452	2.67	12.05	.1962	37.16	49.21	17.99
	[1] 14	29.08	31.85	436	2.79	12.17	.1939	37.55	49.72	17.87
	15	27.55	30.30	418	2.78	11.63	.1919	35.20	46.83	16.53
	[2] 16	28.34	31.10	436	2.79	12.18	.1955	36.89	49.07	17.97

[1] Received an extra amount of phosphorus in the manures.
[2] Received an extra amount of potassium in the manures.
[3] The crop was abnormal in 1909, owing to injury from nematodes.

NOTE.—On an area basis, to calculate from the grams per pot to pounds per acre, multiply by 122.4.

REPORT ON INORGANIC PLANT CONSTITUENTS.

By O. M. SHEDD, *Referee.*

The cooperative work on this subject during the present year has consisted in a comparison of two methods for the separation of ferric and aluminic oxids in an ash solution.

The customary letter asking for cooperation was sent to the various stations in January, and favorable replies were received from 21 analysts, who expressed a desire to assist in the work. To them the directions and samples were sent in February, and results have been received from 10, who have completed all or some part of the work as requested.

COMPOSITION OF SAMPLE.

The sample sent out was a synthetic hydrochloric acid solution of an ash and represented an ash having the following composition, the silica, chlorin, and carbon dioxid not being taken into account:

	Per cent.		Per cent.
K_2O	40.00	Al_2O_3	2.00
Na_2O	3.00	Mn_3O_4	1.00
CaO	23.00	P_2O_5	16.81
MgO	10.00	SO_3	0.50
Fe_2O_3	3.00		

The iron and aluminum used in this solution were obtained from tested chemically pure iron wire and sheet aluminum. The other chemicals used were chemically pure salts, and a control containing all except the iron and aluminum showed that neither of them was present.

METHODS OF ANALYSIS.

The methods and the results obtained by the different analysts who have cooperated in the work are given below.

MOLYBDATE METHOD FOR FERRIC AND ALUMINIC OXIDS.

Use 50 cc aliquots, corresponding to 0.5 gram of ash, for the determination. If there is ferrous iron present, oxidize by boiling with a few cubic centimeters of hydrogen peroxid. Cool the solution, add ammonium hydroxid until a precipitate begins to form, then nitric acid until just clear, and finally add about 2 to 3 cc of concentrated nitric acid in excess. Add 25 cc of ammonium nitrate solution (1:1) free from phosphate, heat to 40° C., and precipitate the phosphomolybdate by adding slowly, with constant shaking, a moderate excess of the official nitric acid molybdate solution.

The temperature of the solution should not at any time exceed 40° C., as a higher temperature has a tendency to precipitate iron and aluminum with the phosphomolybdate.

After the precipitation is made, allow the solution to stand for an hour or two at 40° C., and then for several hours at room temperature, preferably overnight.

After standing for an hour, make sure that sufficient molybdate solution has been added by pipetting 5 cc of the clear solution into an equal volume of the warm reagent. If a precipitate forms, the test portion is to be returned and more molybdate solution added. Filter and wash with about 75 cc of ammonium nitrate solution (2.5 per cent) slightly acidified with nitric acid and free from phosphate, collecting filtrate and washings.

Do not concentrate the solution, because molybdic acid will separate, which will vitiate the results, but cautiously neutralize it in a beaker with ammonium hydroxid, care being taken that the temperature does not rise above 40° C. and that the alkali is added only in very slight excess; allow to stand at the above temperature until the precipitate completely settles. Filter the clear supernatant fluid, wash the precipitate a couple of times with hot water by decantation before transferring it to the filter, and wash four or five times on the filter. Dissolve the precipitate through the filter with weak, hot nitric acid (1:5), wash the filter, and reprecipitate in the same careful manner. The same filter may be used for the second filtration and the volume of the solution for the reprecipitation need not exceed 100 cc. Before the second filtration is made a small quantity of finely divided ashless filter paper pulp is added in order to facilitate the washing and to leave the precipitate finely divided after the ignition so that it can be easily fused with potassium bisulphate for the iron determination. Dry and ignite the precipitate and weigh as ferric and aluminic oxids.

If the precipitate has been treated as indicated the iron oxid can be readily determined as follows: Fuse it with about 4 grams of potassium bisulphate, cool, add 5 cc of concentrated sulphuric acid, and heat to boiling. Transfer to a flask, add water, and digest until all sulphate is dissolved and the solution is clear. Reduce with zinc, cool, and titrate with a fiftieth-normal solution of potassium permanganate. Make blank determinations on chemicals used.

If it is desired to use a larger amount of the sample for the iron determination a suitable aliquot of the original solution can be taken and evaporated directly with sulphuric acid, reduced with zinc, and titrated as described.

OXALATE METHOD FOR FERRIC AND ALUMINIC OXIDS.

Use 100 cc (1 gram) of the sample and, if the iron has not been already oxidized, add a few cubic centimeters of hydrogen peroxid, boil for a few minutes to oxidize the iron and expel oxygen. Add ammonium hydroxid until a precipitate begins to form, then hydrochloric acid until just clear, then 1.5

grams of powdered ammonium oxalate, boil gently for a short time, shaking occasionally to avoid bumping. Let settle, filter and wash the precipitated calcium oxalate. Collect the filtrate and wash water in a 500 cc Kjeldahl flask, add 5 cc of concentrated sulphuric acid, and evaporate to white fumes to destroy excess of oxalic acid. (This boiling down and destruction of oxalic acid can be done in 20 minutes, and during the first part of the boiling care should be taken to prevent bumping, which can be avoided by adding a few small pieces of scrap platinum.) Cool, add 1 gram of ammonium phosphate, 50 cc of water, and 5 cc of concentrated hydrochloric acid. Boil gently a few moments to dissolve any dehydrated ferric sulphate, boiling until the solution is clear, care being taken that the volume is not appreciably reduced. Rinse into a beaker, make up to about 150 cc volume, and precipitate the iron and alumina as phosphates by adding ammonium hydroxid until just alkaline, just clearing with hydrochloric acid, and then adding 25 cc of ammonium acetate solution (sp. gr. 1.04). Or, if preferred, the solution can be treated as in the Kjeldahl flask and poured in a thin stream into the acetate solution, using sufficient water in the rinsing to bring the total volume to that indicated above. A small amount of finely divided ashless filter paper pulp is added to the phosphates, in order to facilitate the washing and to leave them finely divided after the ignition, so that they can be easily fused with potassium bisulphate for the iron determination.

Have both the ammonium acetate solution and the solution containing the phosphates at 80° C. when the two are combined. Heat the phosphates for 10 minutes at 80° C., filter, using suction, and wash with boiling hot ammonium nitrate solution (2.5 per cent) free from phosphate. Carefully ignite the precipitate without removing from the paper, at first with low flame until the paper is charred, gradually increase the heat until all of the carbon is gone, and finally blast for a minute. Deduct the iron phosphate present, calculated from the iron determination, and multiply the remainder by 0.418 to obtain the alumina.

If the precipitate has been treated as indicated, the iron oxid can be readily determined by fusing it with about 4 grams of potassium bisulphate, cooling, adding 5 cc of concentrated sulphuric acid, and heating to boiling. Transfer to a flask, add water, and digest until all sulphate is dissolved and the solution is clear. Reduce with zinc, cool, and titrate with a fiftieth-normal solution of potassium permanganate. Make blank determinations on chemicals used.

If it is desired to use a larger amount of the sample for the iron determination, a suitable aliquot of the original solution can be evaporated directly with sulphuric acid, reduced with zinc, and titrated as just described.

ANALYTICAL RESULTS.

Separation of ferric and aluminic oxids.

[Synthetic ash solution containing 3 per cent Fe_2O_3 and 2 per cent Al_2O_3.]

Analyst.	Molybdate method.			Oxalate method.		
	Ferric oxid.	Aluminic oxid.	Ferric and aluminic oxids.	Ferric oxid.	Aluminic oxid.	Ferric and aluminic oxids.
Laboratory, Stillwell & Gladding, New York City..	....	....	....	[1] 3.07	[1] 2.11	5.15
	....	....	....	[1] 3.00	[1] 2.15	5.19
	....	....	....	....	[1] 1.99	5.03
	....	....	....	3.04	2.08	5.12
G. E. Boltz, Wooster, Ohio...........................	2.98	2.02	5.00	3.02	2.07	5.09
	....	2.06	5.04	2.98	2.05	5.03
	....	2.06	5.04	2.98	2.07	5.05
	....	....	....	2.94	2.19	5.13
	2.98	2.05	5.03	2.98	2.10	5.08

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[1] These results are not included in the general average for ferric and aluminic oxids, because the ferric oxid was obtained by direct evaporation with sulphuric acid.

Separation of ferric and aluminic oxids—Continued.

Analyst.	Molybdate method.			Oxalate method.		
	Ferric oxid.	Aluminic oxid.	Ferric and aluminic oxids.	Ferric oxid.	Aluminic oxid.	Ferric and aluminic oxids.
J. P. Aumer, Urbana, Ill	2.71	2.81	5.52	[1]1.51	[1]0.68	[1]2.19
	2.77	2.43	5.20	[1]1.81	[1]1.03	[1]2.84
	2.74	2.36	5.10	[1]1.38	[1].49	[1]1.87
	2.62	2.40	5.02	[1]1.28	[1].59	[1]1.87
	2.65	2.71	5.36	[1].65	[1].16	[1].81
	......	......	......	[1]1.35	[1].31	[1]1.66
	2.70	2.54	5.24	1.33	.54	1.87
L. T. Bowser, Dayton, Ohio	2.86	2.40	5.26	2.90	[1]3.58	[1]6.48
	2.84	2.48	5.32	2.98	[1]6.16	[1]9.14
	2.91	2.53	5.44	2.65	[1]5.24	[1]7.89
	2.92	2.32	5.24	3.10	[1]3.43	[1]6.53
	2.88	2.43	5.31	2.91	4.60	7.51
A. T. Charron, Ottawa, Canada	[1]1.90	[1]4.82	[1]6.72	[1]1.74	[1]3.65	5.39
	[1]1.94	[1]4.98	[1]6.92	[1]1.60	[1]3.73	5.33
	[1]1.94	[1]4.74	[1]6.68	[1]1.76	[1]3.57	5.33
	1.93	4.85	6.78	1.70	3.65	5.35
W. L. Hadlock, Pullman, Wash	......	......	5.72	......	......	......
	......	......	5.42	......	......	......
	......	......	5.48	......	......	......
	......	......	5.36	......	......	......
	......	......	5.52	......	......	......
	......	......	5.28	......	......	......
	......	......	5.46	......	......	......
P. A. Yoder, Washington, D. C	3.31	2.01	5.32	2.85	2.27	5.12
	3.48	2.18	5.66	2.83	2.12	4.95
	3.45	2.12	5.57	......	......	......
	3.19	2.20	5.39	......	......	......
	3.36	2.13	5.49	2.84	2.19	5.03
L. H. Bailey, Washington, D. C	3.22	1.90	5.12	......	......	......
	3.12	2.10	5.22	......	......	......
	3.17	2.00	5.17	......	......	......
J. F. Breazeale, Washington, D. C	......	......	5.18	......	......	......
O. M. Shedd, Lexington, Ky	3.01	2.13	5.14	2.95	2.32	5.27
	2.95	2.07	5.02	3.01	2.29	5.30
	3.03	2.31	5.34	2.98	2.27	5.25
	2.99	1.85	4.84	3.15	2.19	5.34
	2.97	2.23	5.20	2.82	2.45	5.27
	3.00	2.18	5.18	2.97	2.34	5.31
	3.00	1.90	4.90	3.00	2.42	5.42
	3.12	2.18	5.30	2.93	2.52	5.45
	3.06	2.22	5.28	2.95	2.41	5.36
	3.03	2.15	5.18	2.96	2.38	5.34
	2.92	2.24	5.16	2.97	2.50	5.47
	2.98	2.16	5.14	2.99	2.47	5.46
	2.92	2.26	5.18	2.94	2.40	5.34
	2.95	2.23	5.18	2.97	2.40	5.37
	3.05	1.93	4.98	......	......	......
	3.03	1.79	4.82	......	......	......
	3.00	2.04	5.04	......	......	......
	3.00	2.11	5.11	2.97	2.38	5.35
Number of determinations	33	35	42	24	20	26
General average	2.99	2.20	5.23	2.95	2.31	5.26

[1] Omitted from the general average.

COMMENTS BY ANALYSTS.

Stillwell & Gladding: The ferric oxid was obtained by direct evaporation.

J. P. Aumer: A separate precipitation in the filtrates of the last four of the six determinations, according to the oxalate method, gave the following amounts of ferric and aluminic oxids, respectively: 0.89, 0.74, 0.34, 0.18 per cent; 0.41, 0.38, 0.15, and 0.14 per cent. Further, these second filtrates, upon long standing, still showed phosphate present.

L. T. Bowser: Ferric oxid by direct evaporation with sulphuric acid gave 3.12 per cent and 3.13 per cent.

P. A. Yoder: The following results on ferric oxid were obtained by rereducing the first two solutions of ferric oxid under the molybdate method and retitrating the same. These at first gave 3.31 and 3.48 per cent of ferric oxid. The subsequent retitrations gave 3.13, 3.73, 3.12, and 3.51 per cent of ferric oxid. Retitrations tried on the two solutions under the oxalate method gave 2.88, 2.83, 2.65, and 2.93 per cent of ferric oxid. By direct evaporation of the original ash solution with sulphuric acid, the result obtained was 2.91 per cent of ferric oxid.

L. H. Bailey: Reported that no concordant results could be obtained by the oxalate method.

Note by the referee: Some difficulty seems to have been experienced by a few cooperators in making the iron determination by the bisulphate fusion, undoubtedly due to an incomplete fusion. For this reason, an alternative method for this determination has been added for future work. The last paragraphs of each method for ferric and aluminic oxids, as given in this report, were not included in the methods as sent out for cooperative work, consequently all of the determinations on ferric oxid reported, unless otherwise stated, were made by fusing the precipitate of the double oxids or phosphates with potassium bisulphate.

ADDITIONAL WORK BY THE REFEREES.

In addition, the following results were obtained from W. H. McIntire, of the Pennsylvania State College, who is the associate referee in this work, but they were not received in time to be incorporated in the preceding table. By the molybdate method Mr. McIntire obtained for the sum of the ferric and aluminic oxids: 3.90, 3.90, 3.94, and 4.04 per cent. Average, 3.95 per cent.

Some work has been carried on by the referee on the synthetic solution, extending and applying the molybdate method for the determination of calcium and magnesium in the filtrate from the ammonium hydrate precipitate, disregarding the manganese present. Ordinarily, there will not be as much manganese in an ash as was present in the above solution, and if a determination is desired it can be made separately.

A large amount of work has been done by the referee in perfecting this method, and in the form in which it is now presented excellent results have been obtained by its use. Disregarding the manganese, which does not apparently interfere in an amount as large as that present in the solution, results have been obtained for the calcium and magnesium which agree very closely with the amounts present. As the synthetic solution was prepared chiefly for the iron and aluminium content, small differences in the other bases might be due to impurities, moisture, etc.

It is not the intention of the referee to have the new scheme of analysis take the place of the present one, but rather to supplement it, if after it has been tested by the association it is found satisfactory, as there is no doubt that there will be much time and work saved by using it. The method will have another advantage in that the phosphoric acid, ferric and aluminic oxids, calcium and magnesium oxids, and possibly the manganese can be estimated in the same solution on one-half gram of ash, which is very desirable when the sample is small. Still another advantage which might be mentioned in its favor consists in avoiding the acetate separation, which at best is not very satisfactory in the hands of the average analyst.

There are some additional points that remain to be settled as to the new procedure, one of which is to see if larger amounts of manganese will interfere, and if such is found to be the case, then a separation of this also may be found to work. No work on the separation of the manganese has been tried by the referee, but the associate referee has done some work along this line and obtained some very promising results (p. 66).

The method found by the referee to give the best results in extending the molybdate method for calcium and magnesium is as follows:

Combine the filtrate and washings from the first and second precipitations of ferric and aluminium hydroxids, make strongly alkaline by adding 5 cc of strong ammonium hydroxid, and heat to 100° C. on the water bath. The volume at this point is probably 400–500 cc. Do not concentrate, but add slowly, with constant stirring, an excess of hot ammonium oxalate solution until the calcium is precipitated. Allow the beaker to remain for a few minutes on the water bath, and after the precipitate settles, filter. The time required for the precipitation is about the same as in an ordinary calcium determination.

Dissolve the calcium oxalate in hydrochloric acid, wash the filter, and add a few drops of ammonium oxalate solution. Then add sufficient water to make a volume of about 75–100 cc, heat to 100° C., and reprecipitate the calcium by adding weak ammonium hydroxid slowly with constant shaking until the solution is faintly alkaline.

Combine the filtrates and washings from both precipitations of the calcium and evaporate to about 100 cc, or to as small a volume as possible, so that the salts will remain in solution on cooling. Make the solution slightly acid with hydrochloric acid, add sufficient sodium phosphate solution to precipitate the magnesium and make the whole slightly alkaline with ammonium hydroxid. Stir or shake the solution until the precipitate forms, and then make strongly alkaline by adding 20 cc of strong ammonium hydroxid. Let stand about 24 hours, filter, dissolve the precipitate in hydrochloric acid and reprecipitate in the same manner as before, keeping the volume as small as possible.

Using this method, the following results on the calcium and magnesium were obtained: Calcium oxid, 23.14, 23.48, 23.10, and 23.12 per cent; magnesium oxid, 9.92 and 9.90 per cent.

In working out the method, a number of determinations of the calcium oxid were made by discarding the filtrate from the reprecipitation of the ferric and aluminum hydroxids and not reprecipitating the calcium oxalate.

The results obtained were: Calcium oxid, 23.10, 23.24, 23.26, 23.12, 22.92, 23.12, and 23.02 per cent. A reprecipitation of the calcium oxalate working in the same manner gave 23.06 per cent of calcium oxid. These determinations were made for the purpose of finding out if it was possible to shorten the method.

While the results given are very good, there has been a balancing of errors to a certain extent in that a small amount of calcium is lost in the filtrate discarded, while more or less manganese is present in the first precipitation of the calcium oxalate. It has been the referee's experience that it is necessary to make reprecipitations in every case in order to obtain pure precipitates, and to save both filtrates and the first washings of all precipitates. Especially is this true if a good determination of the magnesium is desired.

The magnesium determination has given the most trouble in this work and several different plans of precipitating the magnesium ammonium phosphate were tried before the best conditions were obtained. The plan was tried of precipitating in a large volume, 800–1,000 cc, with the ammonium salts present as prescribed by Washington in his book on rock analyses. Also, the plan was tried of burning off the ammonium salts carefully, taking up the residue with a small amount of acid, and then precipitating the magnesium in a small volume both by leaving the molybdenum in the solution and also by removing most of it by first filtering the oxids of molybdenum while they were in the acid solution and washing the residue. In all cases the results on the magnesium were too low and the only satisfactory plan found was to leave the ammonium salts in the solution and keep the volume as low as possible so that they would remain in solution during the precipitation of the magnesium ammonium phosphate.

Mr. McIntire, the associate referee, by a method but slightly different from the general method already given, has determined the manganese in addition to the calcium and magnesium in the synthetic solution. The method followed was to precipitate the iron and aluminum hydroxids by the directions given and determine the calcium without concentrating the filtrate by means of ammonium hydroxid and ammonium oxalate. After the elimination of the calcium, the filtrates and washings were evaporated to a small volume and the manganese removed by treatment with bromin water. The filtrate from the manganese was evaporated to about 75 cc and precipitated with sodium phosphate. Following this method the following results were obtained:

Determinations, including manganese, by McIntire.

Aluminic and ferric oxids.	Calcium oxid.	Manganese (Mn_3O_4).	Magnesium oxid.
Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
3.90	24.28	1.16	9.61
3.90	23.88	1.16	9.34
3.94	....	1.28	9.90
4.04	....	....	....

Mr. McIntire suggests that as most plants contain such a small amount of manganese it would be preferable to leave it in the solution and weigh it with the magnesium as the pyrophosphate. The manganese result could then be obtained by dissolving this precipitate and using the sodium bismuthate method.

The referee's results on magnesium seem to indicate that very little if any manganese in this particular solution is precipitated with the magnesium, but this is a point that needs further investigation.

RECOMMENDATIONS.

Following are the recommendations for the present year—

(1) That the molybdate method in the form in which it is now presented for the separation of ferric and aluminic oxids in an ash solution be made official.

(2) That the oxalate method for the above separations be made a provisional method. That further work be done on it next year on a synthetic solution, in order that it may be made an optional method. This method was first suggested by the referee last year, and in a slightly modified form has been published in the Proceedings for 1910.

(3) That cooperative work be done next year on the molybdate method as extended and described for the separation of calcium and magnesium, and probably the manganese in an ash solution.

(4) That cooperative work be done on the method entitled "The Determination of Total Sulphur in Organic Matter," as described by Herman Schreiber in Circular No. 56, Bureau of Chemistry, United States Department of Agriculture. This recommendation is made in order that there may be an optional official method for the determination of total sulphur in plants, which is very desirable.

The work done in this and former years justifies the first two recommendations. The molybdate method has been before the association since 1908, in which year it was recommended by the referee to be made an official method. Since that time 27 analysts have used it at different times in cooperative work for this association, and with very few exceptions have obtained good results.

The second recommendation is made in order that the association may have another method which will be satisfactory for these separations.

The third needs no further comment, while the fourth recommendation, as stated before, is made simply for the reason that it is very desirable to have a check method for the determination of total sulphur in plants.

In addition, Mr. McIntire, the associate referee, desires the association to take some action upon a recommendation which he set forth in a brief report to the chairman of subcommittee A, as follows:

> I would strongly recommend that the method for the preparation of an ash without the use of calcium acetate, which is now an optional official method (Bul. 107, Rev., p. 238), be made the official method for the securing of an ash of different plants, most especially of the cereal plants, because of the fact that there are very minute traces of calcium to be found in these crops, even when grown in the most calcareous soils. In the method calling for the use of calcium acetate, we, of course, are subject to error in the standardization of the chemically pure salt. Afterwards, in making determination of the calcium added to the solution, plus the amount found in the plant, we have a rather large precipitate, a very small percentage of which would make a relatively large error in the actual amount of calcium present in the plant.

The referee is of the opinion that Mr. McIntire's recommendation is a good one in regard to the ashing of a large number of plants, and, furthermore, whatever action is taken by this association, we should, as far as possible, describe the kind of plants to which each method of ashing is best suited.

Three resolutions offered by Mr. Shedd, amending the constitution and by-laws, were referred to Mr. Van Slyke as chairman of the committee on amendments to the constitution (see p. 86).

At 1.40 the convention adjourned until 2 p. m.

MONDAY—AFTERNOON SESSION.

At the opening of the afternoon session the president appointed the following committees:

Committee to invite the Secretary of Agriculture and the Assistant Secretary to address the association: M. E. Jaffa, of California; F. T. Shutt, of Canada; and A. J. Patten, of Michigan.

Committee on resolutions: William Frear, of Pennsylvania; B. B. Ross, of Alabama; and J. G. Lipman, of New Jersey.

Committee on nominations: L. L. Van Slyke, of New York; W. A. Withers, of North Carolina; and B. L. Hartwell, of Rhode Island.

Auditing committee: J. M. Bartlett, of Maine; O. M. Shedd, of Kentucky; and C. W. Stoddart, of Pennsylvania. (B. L. Hartwell, Rhode Island, acting in the absence of Mr. Stoddart.)

REPORT ON INSECTICIDES.

By C. C. McDonnell, *Referee.*

The cooperative work on the subject of insecticides and fungicides this year has consisted of work on methods for the analysis of lead arsenate and lime-sulphur solutions. Reports have been received from five laboratories in addition to the work done in the Insecticide Laboratory of the Bureau of Chemistry.

Lead Arsenate.

The work on this material has been carried out along the lines recommended by the referee and adopted by the association last year. Two samples were submitted for analysis, No. 1 being a commercial sample of lead arsenate paste, and No. 2 a sample prepared in the laboratory from pure chemicals. The determinations of moisture, total lead oxid, and water-soluble arsenic by the following methods were requested:

Moisture.—In case the sample is in the form of a paste (sample No. 1), weigh entire sample and dry at from 80° to 100° C. until dry enough to powder readily, and obtain loss in weight. Powder the dried sample in a mortar and determine the remaining moisture as follows: Dry 2 grams in an air oven at 105° to 110° C. to constant weight. Calculate total moisture from this loss and the loss obtained in the preliminary drying.

On sample No. 2 obtain moisture by drying 2 grams to constant weight at 105° to 110° C.

Lead oxid.—Method I.—See Bureau of Chemistry Bulletin 107, Revised, page 239.

Method II.—Treat 2 grams of the powdered sample with 50 cc of nitric acid (1:4), heat the solution, and when the lead arsenate has dissolved, cool, and make up to 200 cc. Pipette 50 cc of this solution into a 400 cc beaker. dilute to about 300 cc, heat nearly to boiling, add sodium or ammonium hydroxid to incipient precipitation, then add dilute nitric acid (1:10) to redissolve the precipitate, avoiding more than a slight excess; now add to this boiling solution 50 cc of a 10 per cent potassium chromate solution, stirring vigorously during the addition. The chromate solution should be nearly boiling and added to the lead solution by means of a pipette delivering 50 cc in about one minute. If the lead chromate is precipitated hot and stirred vigorously during the precipitation it will settle clear in 15 minutes, or less, when it is ready to be filtered. Filter while hot, collecting the precipitate on a weighed gooch, and wash with boiling distilled water until the final wash water does not show the slightest tinge of yellow. Dry the precipitate in an air oven at 140° to 150° C. to constant weight. (In preparing the filter, the asbestos mat should be thick and pressed down firmly, then washed, and dried at 140° to 150° to constant weight. If porcelain gooch crucibles are used they should be cooled in a desiccator over sulphuric acid, as they take up a little moisture over calcium chlorid.)

Weight of $PbCrO_4 \times 0.6905 = PbO$.

Soluble arsenic.—(1) Method as given in Bureau of Chemistry Bulletin 107, Revised, page 240. Use 500 cc of the water extract for the determination of arsenic. Make correction for the amount of iodin solution required to produce blue color titrated to—using same chemicals and volume as in the determination.

(2) Determine water-soluble arsenic as before, except digest for one day instead of ten.

The results reported by the various analysts are given in the following table:

Cooperative results on lead arsenate.

[Results on lead and arsenic oxids calculated to "moisture-free" basis.]

Analyst.	Sample No. 1.					Sample No. 2.				
	Moisture.	Total lead oxid (PbO).		Water-soluble arsenic oxid (As_2O_5).		Moisture.	Total lead oxid (PbO).		Water-soluble arsenic oxid (As_2O_5).	
				Extraction.					Extraction.	
		Method I.	Method II.	10 days.	1 day.		Method I.	Method II.	10 days.	1 day.
	P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
S. D. Averitt, Lexington, Ky......	40.30	69.17	69.72	1.09	0.85	4.15	62.52	62.67	0.25	0.25
	......	69.30	69.55	1.11	.87	......	62.40	62.65	.28	.25
R. J. Davidson and C. B. Walker,	39.84	69.55	70.13	.99	.60	4.09	61.30	62.75	.20	.12
Blacksburg, Va.	......	69.40	70.11	.94	.60	......	61.41	62.77	.22	.12
	......	69.40	70.06	......	......	......	61.18	......	......	......
C. H. Robinson, Ottawa, Canada...	39.32	69.11	69.45	.78	.59	4.18	62.09	62.84	.14	.12
	......	69.11	69.37	.78	.59	......	62.09	62.67	.14	.12
A. T. Charron, Ottawa, Canada....	......	69.10	68.65	.68	.51	......	62.14	62.55	.14	.11
	......	69.04	68.65	.70	.51	......	62.04	62.54	.14	.11
	......	......	68.56	......	......	......	......	62.56	......	......
Miss M. E. Stover, Berkeley, Cal...	30.26	69.46	68.87	.31	.23	4.24	62.01	62.54	.13	.03
L. T. Bowser, Dayton, Ohio.......	40.04	69.12	......	......	......	......	......	......	......	......
	......	69.22	69.44	1.10	.61	4.13	62.45	62.38	.33	.09
	......	69.44	69.46	.96	.67	4.06	62.41	62.43	.33	.06
	......	69.34	69.62	.99	.61	......	62.48	62.63	......	.06
	......	......	69.59	......	......	......	62.50	62.47	......	......
W. D. Lynch, Washington, D. C...	39.72	69.65	69.44	.70	.48	4.17	62.77	62.88	.07	.03
	......	69.49	69.53	.70	.48	......	62.90	62.82	.06	.03
R. C. Roark, Washington, D. C....	39.51	69.43	69.69	.81	.68	3.97	62.19	62.91	.09	.05
	......	69.29	69.62	......	......	......	62.30	62.95	......	......
	......	69.16	69.41	......	......	......	......	62.83	......	......
C. C. McDonnell, Washington, D. C.	40.07	69.38	69.70	.70	.60	4.13	62.44	62.89	.08	.06
	......	69.65	69.75	.70	.60	......	62.54	62.88	.08	.06
Average......................	39.76	69.32	69.47	.83	.59	4.12	62.21	62.70	.17	.10

Mr. Averitt, the associate referee, reports Method II for total lead as "easy to work, rapid, and accurate." For soluble arsenic he suggests shaking every quarter hour during a working day and filtering after standing overnight. Mr. Bowser reports "the chromate method seems very good, and I am making use of it on other work to advantage." He suggests that acid and alkali of known strength be used in preparing the solution in order to avoid getting too large an excess of nitric acid before precipitating with the chromate solution.

The results on total lead oxid by the sulphate method agree well. Neither of the samples contains calcium salts, which would render this method unreliable. The results by the chromate method do not agree quite so well, but in consideration of the fact that it is the first time it has been submitted for trial they can be considered quite satisfactory. The point about the method which demands care is to avoid leaving more than a slight excess of nitric acid when making the precipitation. By using dilute acid and adding it carefully there need be no trouble from this cause. It has been found in this laboratory that ammonium hydroxid is a little more desirable for neutralizing excess acid than sodium hydroxid; the lead chromate precipitate collects better, and slightly more satisfactory results are obtained. This method has been in use in this laboratory for the past two years and has always given satisfactory results. I have no hesitancy in recommending its adoption as an official method.

The results on water-soluble arsenic are not entirely satisfactory. In the carrying out of the method there are several points which must be observed. In the first place, in filtering the solution after digesting the lead arsenate with water certain samples have a tendency to run through the filter, and in some cases it is almost impossible to obtain a clear filtrate. We have found that by shaking up the sample just before filtering a clear filtrate can usually be obtained after discarding the first portion that comes through. The temperature of the water during the digestion has a slight influence on the amount dissolved. Great care must be exercised in the addition of thiosulphate and in the final titration with iodin. Where the amount of arsenic present is so small, an error of a drop or two of either of these solutions will make a marked difference in the results. In view of the determinations reported and awaiting the results of other work which we are now doing relative to time of digestion, temperature, etc., no recommendation will be made at this time.

The results on moisture show a satisfactory agreement.

Lime-Sulphur Solutions.

On account of the necessity of having official methods for the determination of the various sulphur compounds in lime-sulphur solutions, those here given were submitted for trial. They are, with slight modifications, the same as given in Bureau of Chemistry Bulletin 101, page 9, which have been adapted from methods given in Sutton's Volumetric Analysis, and Avery's method for total sulphur as adopted by the association in 1909:

Total sulphur in solution.—Bureau of Chemistry Bulletin 107, Revised, page 34. Instead of measuring 10 cc of the sample, weigh accurately about 10 grams and make up to 100 cc. Use 10 cc aliquots of this for each determination.

Sulphur as sulphids.—Dilute 10 cc of the solution, prepared as for total sulphur, to about 100 cc and add ammoniacal zinc chlorid solution (prepared by dissolving 50 grams pure zinc chlorid in water and adding ammonia in sufficient quantity to redissolve the precipitation first formed) until the sulphid is all precipitated, as will be shown by adding a drop of the clear solution to a few drops of nickel sulphate solution. Place on steam bath and heat until the odor of ammonia becomes faint, then filter and wash the precipitate. Transfer filter containing the zinc sulphid precipitate to a beaker, add 10 to 15 cc of a saturated solution of sodium or potassium hydroxid and heat on steam bath for about 15 minutes. Dilute with about an equal quantity of water, add 50 cc hydrogen peroxid solution, and heat on steam bath for 30 minutes. Then make the solution slightly acid with hydrochloric acid, filter to remove paper, wash thoroughly, heat to boiling, and precipitate the sulphate with barium chlorid solution. Calculate sulphid sulphur from the weight of barium sulphate obtained.

(Make blank determinations of sulphur (SO_3) in the reagents used in the determinations of total and sulphid sulphur and make correction therefor. If the hydrogen peroxid solution is not full strength, 50 cc may not be sufficient to oxidize all the sulphur compounds to sulphate, in which case more peroxid must be used.)

Sulphur as thiosulphate.—Dilute 20 cc of the solution prepared as for total sulphur to about 50 cc in a 200 cc graduated flask. Add ammoniacal zinc chlorid until in slight excess and make to mark. Shake thoroughly and filter through a dry filter. To 100 cc of the filtrate add methyl orange and exactly neutralize with dilute hydrochloric acid. Titrate this solution with tenth-normal iodin, using a few drops of starch paste as indicator. From the amount of iodin solution required calculate the sulphur present as thiosulphate, as represented by the following reaction: $2Na_2S_2O_3+2I=2NaI+Na_2S_4O_6$.

Sulphur as sulphate and sulphite.—To the solution from the determination of thiosulphate add two or three drops of hydrochloric acid, heat to boiling, precipitate with barium chlorid solution, and obtain weight of barium sulphate, from which calculate sulphur and report as "sulphate and sulphite sulphur."

Note.—In case sulphite is present the determination of thiosulphate will be too high. However, as calcium sulphite is nearly insoluble it will not be present in more than traces, and the error from this cause will be negligible. We have never examined a sample of lime sulphur in which the combined sulphate and sulphite amounted to more than a small fraction of a per cent.

Two samples were submitted for the work, which were prepared as follows: Weighed amounts of lime, sulphur, and water were boiled together, and two measured portions of the clear solution thus obtained were used. No. 1 was diluted to a definite volume with water and No. 2 was diluted to the same volume with a solution containing a weighed amount of sodium thiosulphate, from which, by calculation, sample No. 2 contained 2.02 per cent of sulphur as thiosulphate in excess of that in No. 1. Owing to the difference in the specific gravity of the two solutions the sulphid sulphur in No. 2 should be 0.4 per cent lower than in No. 1, assuming that no chemical change has taken place in the samples.

Cooperative results on lime-sulphur.

Analyst.	Sample No. 1.				Sample No. 2.			
	Total sulphur.	Sulphid sulphur.	Thiosulphate sulphur.	Sulphate sulphur.	Total sulphur.	Sulphid sulphur.	Thiosulphate sulphur.	Sulphate sulphur.
	P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
R. [1] Davidson and C. B. Walker, Blacksburg, Va.	12.48	[1] 10.78	1.85	......	[1] 14.84	10.12	3.82	......
	12.49	10.78	1.85		14.86	10.18	3.82	
	12.49	10.80	1.85		14.33	10.33	3.82	
	12.46							
S. D. Averitt Lexington, Ky.	12.28	10.19	1.80	0.03	13.75	10.32	3.75	0.03
	12.23	10.33	1.87	.02	13.82	10.50	3.83	.02
	12.28	......	1.81	.04	13.74	......	3.79	.04
	......	......	1.84	......	......	......	3.83	......
	......	......	1.81	......	......	......	3.79	......
A. T. Charron, Ottawa, Canada	12.50	10.33	1.84	.09	14.12	9.85	3.80	.24
	12.49	10.32	1.84	.09	14.06	9.86	3.80	.26
	12.47	......	......	......	14.14	......	......	......
C. H. Robinson, Ottawa, Canada	12.51	10.39	1.84	......	14.14	9.83	3.77	......
	12.44	10.36	1.84	......	14.06	9.84	3.77	......
M. E. Stover, Berkeley, Cal.	12.45	10.47	1.91	.04	14.13	9.96	4.09	.08
R. C. Roark, Washington, D. C.	12.22	10.59	1.80	.16	14.02	10.26	3.73	.12
	12.51	10.57	1.80	.13	14.07	10.44	3.63	.12
W. D. Lynch, Washington, D. C.	12.48	10.48	1.80	.04	14.07	10.36	3.84	.06
	12.45	10.42	1.82	.06	14.11	10.34	3.82	.08
	......	......	......	......	......	10.29	3.81	......
C. C. McDonnell, Washington, D. C.	12.14	10.40	1.83	.02	14.11	10.11	3.81	.05
	12.15	10.32	1.84	.03	......	10.10	3.92	.06
Average	12.40	10.40	1.83	.06	14.02	10.16	3.81	.10

[1] Not included in average; no blank run on reagents used.

The results on the lime-sulphur samples are good, as a whole. The thiosulphate determinations are very close and the work shows the method to be accurate. Practically the theoretical amount added to No. 2 is accounted for.

In the determination of sulphate, the solution must not be boiled, because by so doing some sulphate will be formed from decomposition of the tetrathionate, particularly if more than a trace of hydrochloric acid is present and the solution contains a considerable amount of tetrathionate, as in sample No. 2.

In making the precipitation, warm the solution on a steam bath, then add the barium chlorid, stirring the solution well for several minutes, and let stand in the cold overnight before filtering.

RECOMMENDATIONS.

It is recommended—

(1) That the chromate method (Method II) for total lead oxid in lead arsenate be adopted as official.

(2) That the method for water-soluble arsenic oxid in lead arsenate be further studied as regards time of standing for the solution of soluble arsenic and the effect of different temperatures thereon.

(3) That the method for total sulphur in lime-sulphur solutions (Bureau of Chemistry Bul. 107, Rev., p. 34) be changed as follows: Under "2," line 1, after "Measure," insert "and accurately weigh;" after "sample" strike out "in" and insert a comma and the words "transfer to," and the method as thus changed be adopted as official. (By both measuring and weighing the

sample operated upon, the "per cent by weight" and "grams per 100 cc" of the determined constituents can readily be calculated.)

(4) That the gravimetric method for sulphur as sulphids and polysulphids in lime-sulphur solutions, as given in this report, be adopted as official.

(5) That the volumetric method for sulphur occurring as thiosulphate in lime-sulphur solutions, as given in this report, be adopted as official.

(6) That the method here given for sulphur occurring as sulphates and sulphites in lime-sulphur solutions be changed as follows: Strike out "heat to boiling, etc.," to end of paragraph, and substitute "warm on steam bath, precipitate with barium chlorid solution, stirring vigorously for several minutes, let stand in the cold overnight, filter and obtain weight of barium sulphate. From this weight calculate sulphur," and that the method as changed be adopted as official.

The following recommendations which were adopted by the association in 1909 are hereby renewed for final action.

(7) That "Method II" for total arsenious oxid in London purple, as given in Bulletin 107, Revised, page 29, be dropped from the methods of analysis.

(8) That "Method II" for total arsenic oxid in London purple, as given in Bulletin 107, Revised, page 29, be dropped from the methods of analysis.

The following recommendations adopted by the association in 1910 are hereby renewed for final action:

(9) That "Method I" for total arsenious oxid in London purple, as given in Bulletin 107, Revised, page 28, be adopted as official.

(10) That "Method III" as proposed by the referee in 1909 (Bul. 132, p. 43) for the total arsenic oxid in London purple be adopted as official and designated as "Method II."

(11) That the Gatehouse method (Sutton's Volumetric Analysis, 9th ed., p. 201; Bureau of Chemistry Cir. 10, Rev., p. 6) for the determination of chlorin in cyanids be adopted as official.

(12) That the provisional methods for the analysis of lead arsenate (Bul. 107, Rev., p. 239) be changed in accordance with recommendation 7 of the referee in 1910 (Bul. 137, p. 47), and as changed be adopted as official. (The additions and changes as recommended were adopted as provisional in 1910.)

The secretary-treasurer of the association presented his report, which was referred to the auditing committee (see p. 212). He also presented the formal invitation of the officers and members of the executive committee of the Eighth International Congress of Applied Chemistry to the association to join the congress and take part in the proceedings, the opening meeting to be held in Washington, September 4, and the subsequent meetings in New York, closing on September 13. Reply to be addressed to Bernhard C. Hesse, 25 Broad Street, New York.

A resolution by Mr. Withers in regard to the appointment of referees by the outgoing executive committee was referred to the committee on amendments to the constitution (see p. 86).

REPORT ON WATER.

By W. W. Skinner, *Referee.*

The cooperative work on water analysis for this year has been a continuation of the plan of work prosecuted the year previous with a very slight modification in the several methods used. The work, as heretofore, has been restricted to a consideration of the more common determinations in mineral water analysis, strontium being the only determination which has been added to the work, while lithium has been omitted because of the excellent results of last year obtained by the Gooch method, and partly because to have included it in this year's determinations would probably have made the cooperative work on water rather tediously long. A general letter of invitation to participate in the work was sent to numerous chemists known to be interested in water analysis, 16 of whom responded with a request that samples and directions for the work be forwarded. Ten chemists have submitted results in time to have them incorporated in the report. It was found to be practically impossible to obtain a natural water which would contain the several constituents in sufficient amounts to give a thorough test of the several methods proposed for their determination. It seemed desirable on account of the size of the sample also to have the concentration such that not in excess of 1 liter should be taken for any one determination. With these points in mind, a sample of water was prepared, the basis of which was filtered Potomac River water to which were added known quantities of several salts in which the natural water for our purposes was deemed lacking. The sample after being prepared was allowed to remain in the laboratory for several days with occasional agitation. It was then transferred to the several packages and immediately sent by express to the collaborators, with instructions that the determinations of bicarbonic acid, nitric acid, and chlorin be made as soon after the sample was received as possible, and then if it were necessary to delay the work, that the several portions should be withdrawn for the determinations and acidified with hydrochloric acid in order to prevent any possible action of the water upon the glass container. The following table gives the results obtained by the several analysts. In order to avoid the introduction of any personal element into the report, the names of the analysts have been omitted.

Analysis No. 1 was made in the water laboratory of the Bureau of Chemistry immediately after the sample was prepared and at about the time it was supposed that other analysts would be engaged in the cooperative work.

Analysis No. 2 was made in the water laboratory after the sample had stood in the glass container for six months. It was made by another chemist working independently, who was unaware of the results of analysis No. 1. A comparison of these two analyses shows that the nitric acid, aluminum, and silica are slightly greater in analysis No. 2 where the water had been allowed to stand in the container for a period of six months. The other figures are in reasonably close agreement.

A comparison of the results given in the table for sulphuric acid shows, with one exception, a reasonably close agreement. This exception is in the case of analysis No. 8. It should be noted further, however, that the results submitted by No. 8 are generally incorrect and have been largely omitted from the general average. The agreement would seem to indicate that the technique employed in the determination of sulphates is correct. When, however, sulphates are high, accompanied by a high content of sodium, the results for sulphates are generally too low. This error in the determination of sulphates was pointed out by Allen and Johnston,[1] and by Johnston and Adams[2], and it is advisable for waters high in these salts to modify the method as suggested by the latter.

The results for bicarbonic acid vary to a considerable extent, but with one exception are within what might be considered a reasonable agreement. The variation in this determination is probably due to the difficulty in reading the end point with methyl orange. It has been our experience that this color change probably causes greater difficulty and uncertainty than any other determination. Part of the difficulty may possibly be attributed to inferior methyl orange used, as in our laboratory we have had to reject numerous samples of methyl orange before obtaining a supply reasonably sensitive in its reaction.

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[1] J. Amer. Chem. Soc., 1910, 32: 588.

[2] J. Amer. Chem. Soc., 1911, 33: 829.

The results for the determination of nitric acid are very unsatisfactory. It will be noticed that in the two analyses made in this bureau, No. 2 some six months after No. 1, the nitric acid had increased over one part per million. This increase was probably due to the nitrification of the small amount of organic matter contained in the Potomac River water. The results, however, obtained by analysts 4, 5, and 7 are entirely unexplainable. The variation in this determination seems to be a very serious consideration when it is remembered that in a sanitary analysis the judgment of the character of a water supply is often dependent upon the report on the content of nitrate. It is possible that some of the difficulty may be attributed to the use of potassium hydroxid as a neutralizing agent in the place of ammonium hydroxid, as suggested by Chamot. If with this modification the silver salt is not entirely removed in the reaction, incorrect results are apt to occur. For this reason the referee is of the opinion that where the use of ammonium hydroxid is not a serious objection to general laboratory work nothing is to be gained by the substitution of potassium hydroxid for ammonium hydroxid in this determination.

For such a fundamental determination as chlorin a very close agreement would be expected. That such, however, is not the case seems to the referee to be a reflection rather upon the worker than upon the method. The actual chlorin in the sample of water is known to be between 62 and 63 parts per million, and that one analyst should obtain a determination of 73.5 parts and one other a determination of 69, and two others determinations of 67.5 and 67.4, respectively, seems rather astonishing. Using a standard solution of silver nitrate, 1 cc equivalent to 1 mg of chlorin, the 10 parts per million in excess obtained by No. 8 is equivalent to a difference of 1 cc in the titration on 100 cc of water, when the total titration consumed only between 6 and 7 cc. If a weaker solution of silver nitrate is used, i. e., 1 cc equivalent to 0.5 mg chlorin, the difference in the titration is equivalent to 2 cc. In view of the fact that this weaker solution can be read within 0.2 or 0.3 cc with accuracy, this variation would seem to point to a lack of care in preparing the standard solution.

The figures for iron and aluminum are not of very great significance and are approximately in agreement with the amount of these elements known to exist in Potomac River water. The slight increase shown in analysis No. 2 over that of No. 1 is possibly to be explained by contamination from the glass container, possibly silica.

The determinations of calcium are, with the exception of analyses Nos. 6, 8, and 9, fairly satisfactory. It should be noted, however, that No. 9 reports no strontium, while it is known that 3.5 parts per million are present; if the strontium is deducted from the report on calcium it would bring the results in fairly close agreement with the average. The determinations reported in analysis No. 8 are high, but as the strontium is low, it is evident that the calcium figure is contaminated with strontium. However, if the known amount of strontium were subtracted, the result for calcium would still be high. Analysis No. 6 shows 41.2 parts of calcium, but no strontium. Deducting the 3.5 of strontium known to be present from the 41.2 of calcium reported makes the result quite low.

The results on strontium are unsatisfactory and are to be attributed, possibly, to a lack of experience in this determination. The water was known to contain exactly 3.5 parts per million of strontium. It will be noted that of the eight analyses reporting the determination of strontium four had to be omitted from the average because of the wide variation. The method for strontium is as follows:

Dissolve the above oxids (calcium and strontium) with dilute nitric acid and test with the spectroscope for strontium. If strontium is found transfer the nitric acid solution to a small Erlenmeyer flask and evaporate to complete dryness on the steam bath. Heat at 150° to 160° for one or two hours until perfectly dry. Cool, add from 3 to 5 cc of a mixture of equal parts of absolute alcohol and ether. Cork the flask and allow to stand for 12 hours, with occasional shaking. Filter, wash with alcohol ether mixture until a few drops of the filtrate evaporated on platinum foil or watch glass leaves no residue. Evaporate the filtrate to dryness. Dissolve the calcium nitrate in water, precipitate as oxalate, filter, wash, ignite. and weigh as calcium oxid.

Dry the paper and precipitate. Dissolve the strontium nitrate with a few cubic centimeters of hot water. Add a few drops of sulphuric acid, then a volume of alcohol equai to the volume of the solution. Allow to stand 12 hours. Filter, ignite, and weigh as strontium sulphate. Test spectroscopically for absence of calcium.

This method has been very carefully studied in the water laboratory of the Bureau of Chemistry; it has, with proper care, given most excellent results, and is considered much superior to the older ammonium sulphate method originally proposed.

The results for magnesium are fairly satisfactory, with the exception of the figures reported in analyses Nos. 4 and 5. These results are entirely too low and have been omitted from the average.

The data on potassium are satisfactory, with the exception of the results reported by analyst No. 8. In view of the peculiar coincidence in the arrangement of the figures in the report of analyst No. 8, he was communicated with to determine if a typographical error had been made. In reply he stated that the results as reported were obtained. No explanation can be offered for a result of this character.

The figures on sodium are satisfactory, while those for silica show slight variations which may be attributed in some cases to the action of the water upon the container. Thus the increase from 5.2 to 7.9 between analyses Nos. 1 and 2, where it was known that the water had remained in the glass container for a period of six months indicates a decided solvent action of the water upon the glass.

Results of cooperative work on water.

[Ions in parts per million.]

Determination.	Analyst.							
	No. 1.		No. 2.		No. 3.		No. 4.	
	Ions.	Milligram equivalent.	Ions.	Milligram equivalent.	Ions.	Milligram equivalent.	Ions.	Milligram equivalent.
Sulphuric acid (SO_4)	72.0	−1.499	72.1	−1.501	73.25	−1.525	71.8	−1.495
Bicarbonic acid (HCO_3)	173.3	−2.841	181.4	−2.973	178.42	−2.924	[1] 201.6	−3.304
Nitric acid (NO_3)	3.2	− .052	4.4	− .071	4.54	− .073	[1] 11.25	− .181
Chlorin (Cl)	62.0	−1.748	62.5	−1.762	62.75	−1.770	65.2	−1.839
Iron (Fe)	.2	+ .011	.4	+ .021	.50	+ .027	1.0	+ .054
Aluminum (Al)	.5	....	1.7	....	....	....	.0	....
Calcium (Ca)	40.8	+2.035	40.3	+2.010	42.24	+2.107	39.4	+1.966
Strontium (Sr)	3.4	+ .078	3.1	+ .071	2.51	+ .057	[1] 1.9	+ .043
Magnesium (Mg)	19.3	+1.587	19.2	+1.579	19.80	+1.628	[1] 13.19	+1.085
Potassium (K)	12.4	+ .317	12.9	+ .330	12.79	+ .327	13.26	+ .339
Sodium (Na)	52.4	+2.278	51.6	+2.243	54.47	+2.368	49.45	+2.150
Silica (SiO_2)	5.2	....	7.9	....	4.32	....	4.0	....
Total	444.7	....	457.5	....	455.59	....	472.05	....
Sum of plus milligram equivalents	......	+6.306	......	+6.254	......	+6.514	......	+5.695
Sum of minus milligram equivalents	......	−6.140	......	−6.307	......	−6.292	......	−6.819
Difference	......	+ .166	......	− .053	......	+ .222	......	−1.124

[1] Omitted from average.

Results of cooperative work on water—Continued.

Determination.	Analyst.							
	No. 5.		No. 6.		No. 7.		No. 8.	
	Ions.	Milligram equivalent.	Ions.	Milligram equivalent.	Ions.	Milligram equivalent.	Ions.	Milligram equivalent.
Sulphuric acid (SO_4)	72.8	−1.516	72.00	−1.499	73.0	−1.520	[1] 77.46	−1.612
Bicarbonic acid (HCO_3)	176.9	−2.900	175.56	−2.878	178.4	−2.924	173.85	−2.850
Nitric acid (NO_3)	[1] 1.4	− .022	3.18	− .051	[1] .29	− .005	4.25	− .068
Chlorin (Cl)	67.5	−1.904	62.50	−1.762	67.4	−1.901	[1] 73.50	−2.073
Iron (Fe)	.33	+ .018	.40	+ .021	.91	+ .049	.65	+ .035
Aluminum (Al)	.86	....	....	....	....	....	.35	....
Calcium (Ca)	40.6	+2.025	[1] 41.22	+2.056	37.3	+1.861	[1] 46.53	+2.321
Strontium (Sr)	[1] 1.6	+ .036	.00	.000	[1] 5.3	+ .121	[1] 1.67	+ .038
Magnesium (Mg)	[1] 14.9	+1.225	18.95	+1.558	18.3	+1.505	17.15	+1.410
Potassium (K)	11.7	+ .299	13.65	+ .349	12.4	+ .317	[1] 21.97	+ .562
Sodium (Na)	54.1	+2.352	51.45	+2.237	54.1	+2.352	48.61	+2.113
Silica (SiO_2)	4.8	....	6.85	....	5.7	....	7.33	....
Total	447.49	....	445.76	....	453.10	....	473.32	....
Sum of plus milligram equivalents	....	+5.955	....	+6.221	....	+6.205	....	+6.479
Sum of minus milligram equivalents	....	−6.342	....	−6.190	....	−6.350	....	−6.603
Difference	....	− .387	....	+ .031	....	− .145	....	− .124

Determination.	Analyst.				Extremes and means.		
	No. 9.		No. 10.		Highest.	Lowest.	Average.
	Ions.	Milligram equivalent.	Ions.	Milligram equivalent.			
Sulphuric acid (SO_4)	72.9	−1.518	71.61	−1.491	73.25	71.61	72.4
Bicarbonic acid (HCO_3)	[1] 188.4	−3.088	176.90	−2.900	184.5	173.3	178.9
Nitric acid (NO_3)	....	....	4.37	− .070	4.54	3.18	3.9
Chlorin (Cl)	63.93	−1.803	69.15	−1.950	69.15	62.0	65.6
Iron (Fe)	.22	+ .012	.66	+ .035	2.1	.4	1.2
Aluminum (Al)	.59	....	....	....			
Calcium (Ca)	[1] 45.10	+2.251	41.09	+2.050	42.24	37.3	39.8
Strontium (Sr)	....	....	2.95	+ .067	3.4	2.51	3.0
Magnesium (Mg)	17.84	+1.467	19.33	+1.590	19.8	17.15	18.5
Potassium (K)	12.86	+ .329	11.27	+ .288	13.65	11.27	12.5
Sodium (Na)	50.93	+2.216	52.70	+2.291	54.47	48.61	51.5
Silica (SiO_2)	3.8	....	4.80	....	7.9	3.8	5.8
Total	456.62	....	454.83	....	475.0	431.13	453.1
Sum of plus milligram equivalents	....	+6.275	....	+6.321	....	....	....
Sum of minus milligram equivalents	....	−6.409	....	−6.411	....	....	....
Difference	....	− .134	....	− .090	....	....	....

[1] Omitted from average.

In order to show the balance of each analysis and bring out more clearly the variations in the comparison, there has been included in the table the milligram-equivalent of each determination with its proper sign. If the analysis is properly balanced, that is, if the basic and acid elements are in proper proportion, the difference between the plus and minus equivalents should be small. The obtaining of this balance in equivalents is a useful method when properly employed for detecting possible errors in a complete mineral analysis.

It also gives more clearly a thorough appreciation of the error of the analysis. Thus, by referring to the chart, it will be noticed that the errors in analyses

Nos. 1 and 2 were +0.166 and —0.053, respectively. This indicates that the algebraic sum of the errors in analysis No. 1 is equivalent to approximately 1.35 per cent, and the sum of the errors in analysis 2 is equivalent to an error of approximately 0.41 per cent. Such balances are to be considered very satisfactory in water of a mineralization similar to this. An inspection of the chart will show that analysis No. 4 is seriously out of balance and should, therefore, be entirely rejected from consideration. In the statement of the general averages and also of the maximum and minimum figures, however, the results of No. 4 have been omitted only where they are shown to be very wide in their variation from the other determinations. While a large plus or minus difference in the algebraic sum of the equivalents indicates that there is a serious error in the analysis, a slight difference does not, on the contrary, always indicate that the analysis is correct, because it is entirely possible for the errors to occur in such manner as to compensate for each other. Thus, in analysis No. 8, in which 5 of the 12 determinations had to be omitted from the general average because of the wide variation, it is noted that the difference is only —0.085 in the balance of the equivalents.

The results of the cooperative work this year, as a whole, are decidedly an improvement over those obtained last year. While numerous results, and in two instances almost the entire analysis, had to be omitted from consideration, the reports are fairly satsfactory and indicate that experience with the methods is all that is necessary to obtain accurate results. While collaborative work is to some extent a test of the men doing it, the primary object, of course, is to arrive at a correct estimate of a proposed method. It is of the highest importance, therefore, that the work submitted has received that painstaking care which its importance warrants.

RECOMMENDATIONS.

It is respectfully recommended that the methods for water analysis proposed in Circular 52 be adopted as provisional methods of the association.

[The following modifications of these methods as printed in Circular 52 are offered for adoption in 1912.]

MODIFIED METHODS FOR THE ANALYSIS OF MINERAL WATERS.

1. TOTAL SALTS IN SOLUTION.

[As in Circular 52, page 6.]

2. LOSS ON IGNITION.

[As in Circular 52, page 6.]

3. SILICA, IRON, ALUMINUM, MANGANESE, CALCIUM, STRONTIUM, MAGNESIUM.

A preliminary examination should be made using from 100 to 250 cc of water to determine the quantity of calcium and magnesium present, which determines the quantity of water to be evaporated for the final analysis.

(*a*) *Silica.*

Evaporate such quantity of the water that the weight of calcium oxid will be from 0.1 to 0.6 gram or the weight of magnesium pyrophosphate 0.1 to 1.0 gram. Acidify the quantity of water (usually from 1 to 5 liters) with hydrochloric acid and evaporate to dryness in a platinum dish; continue the drying on the water bath for about 1 hour. Drench the residue with hydrochloric acid (usually 5 to 15 cc are sufficient). Allow to stand 10 to 15 minutes and add sufficient water to bring the salts into solution. Heat on the steam bath until solution of the salt is effected. Filter and wash thoroughly with hot water. This removes most of the silica. Evaporate the filtrate to dryness; take up with 5 or 10 cc of hydrochloric acid and sufficient water as before. Heat, filter, and wash thoroughly with hot water. The filtrate is solution A. Transfer the two residues to a platinum crucible, ignite, blast, and weigh. Moisten the contents of the crucible with a few drops of water; add a few drops of sulphuric acid and a few cubic centimeters of hydrofluoric acid and evaporate on the water bath under a good hood. Repeat the treatment if all silica is not volatilized. Dry carefully on a hot plate, ignite, blast, and weigh. The difference is silica. The residue in the crucible, usually alumina and iron oxid, is added to the total iron and aluminum oxids later in the analysis.

(*b*) *Iron and aluminum.*

Concentrate solution A to about 200 cc; while still hot add ammonium hydroxid slowly with constant stirring until it can be faintly smelled coming off from the solution. Boil the solution until the smell of ammonia has nearly but not quite disappeared. Filter and wash two or three times with hot water. Dissolve the precipitate in hot hydrochloric acid. Make to a volume of approximately 25 cc, boil and again precipitate with ammonium hydroxid; filter, wash thoroughly with hot water, dry, ignite, and weigh as iron and aluminum oxids.[1] The filtrate is solution B.

(*c*) *Iron.*

Fuse the residue of iron and aluminum oxids with fused potassium hydrogen sulphate. This fusion takes but a few minutes and must not be continued beyond the time actually needed. When the fusion is complete it is set aside and allowed to cool. Add dilute sulphuric acid and heat the crucible until the fusion is dissolved. Evaporate on the water bath as far as possible; then heat gradually until fumes of sulphuric acid come off copiously. Dissolve in water and allow to stand on the water bath. Cool the solution, transfer to an Erlenmeyer flask, and make up to such a volume that it does not contain more than 2.5 per cent of free sulphuric acid. Hydrogen sulphid[2] is passed through the solution reducing the iron and precipitating the platinum contaminating the residue from the fusion. Filter and wash. Again pass through the solution hydrogen sulphid. Be sure that all the iron is reduced. Next expel the hydrogen sulphid by boiling, but at the same time pass through the solution a current of carbon dioxid, the removal of all hydrogen sulphid being found by occasionally testing the escaping gas with lead acetate paper. When hydrogen sulphid has been removed boiling is discontinued, the flask allowed to cool somewhat without discontinuing the current of carbon dioxid. The reduced iron is then titrated with a standard permanganate solution and calculated as iron.

If the amount of iron is less than 1 mg it may be determined colorimetrically, as follows:

Fuse the ignited precipitate of iron and aluminum oxids with potassium hydrogen sulphate, dissolve in water, and precipitate the iron and aluminum with ammonium hydroxid. Dissolve the precipitate from the filter paper in hydrochloric and nitric acids, dilute the solution, add ammonium sulphocyanate, and compare the color developed with that of calibrated color disks, or standards containing known amounts of iron.

(*d*) *Aluminum.*

Subtract the iron found as oxid from the original weight of iron and aluminum oxid, which in the absence of phosphates gives the weight of aluminum oxid. Calculate to aluminum.

(*e*) *Colorimetric determination of manganese.*

To from 50 to 100 cc of the water in a beaker add about 1 cc of strong nitric acid and approximately 0.02 gram of silver nitrate (10 cc of a solution of 2 grams per liter). If a precipitate of silver chlorid appears, more silver nitrate must be added until the chlorin is precipitated and as much as 0.02 gram of silver nitrate remains in solution. Filter from the silver chlorid, add 1 gram of ammonium persulphate to the filtrate, and place the beaker or flask containing the solution on the steam bath until a pink color develops (usually about 20 minutes). Compare the color developed with standards prepared by treating with nitric acid and ammonium persulphate solutions containing known amounts of manganese. To prepare a standard solution of manganese sulphate, weigh out 0.2877 gram of pure potassium permanganate, dissolve in a small amount of water, and add an excess of sulphuric acid; carefully reduce with oxalic acid and make up to 1 liter; 1 cc of this solution contains 0.0001 gram of manganese. If only a small quantity of manganese is present, determine it in the sodium carbonate precipitate which was extracted for bromin, iodin, arsenic, and boron (as given on p. 9, Bureau of Chemistry Circular 52). Dissolve the precipitate in nitric acid and treat with silver nitrate and ammonium persulphate, as given above.

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[1] This is in the absence of phosphates. If phosphoric acid is present in small amounts it is precipitated with the iron and aluminum.

[2] Zinc may be used instead of hydrogen sulphid for reducing the iron.

(*f*) *Calcium, strontium, magnesium.*

Concentrate solution B to about 150 or 200 cc, and to this solution containing not more than 0.6 gram of calcium oxid or 1 gram of magnesium, calculated as pyrophosphate, add from 1 to 2 grams of oxalic acid and sufficient hydrochloric acid to clear the solution. Heat to boiling and neutralize with ammonium hydroxid, constantly stirring. Add ammonium hydroxid in slight excess and allow to stand for three hours, preferably in a warm place. Filter off the supernatant liquid and wash the precipitate by decantation once or twice with cold water. Dissolve precipitate with hydrochloric acid, dilute to from 100 to 200 cc, add a little oxalic acid, and precipitate as before. After standing three hours filter, wash with cold water,[1] dry, ignite, and blast and weigh as calcium and strontium oxids. The filtrate and washings are solution C.

Dissolve the above oxids with dilute nitric acid and test with the spectroscope for strontium. If strontium is found, transfer the nitric-acid solution to a small Erlenmeyer flask and evaporate to complete dryness on the steam bath. Heat at 150° to 160° C. for one or two hours until perfectly dry. Cool, add from 3 to 5 cc of a mixture of equal parts of absolute alcohol and ether. Cork the flask and allow to stand for 12 hours, with occasional shaking. Filter, wash with alcohol-ether mixture until a few drops of the filtrate evaporated on platinum foil or watch glass leaves no residue.

Dry the paper and precipitate. Dissolve the strontium nitrate with a few cubic centimeters of hot water. Add a few drops of sulphuric acid, then a volume of alcohol equal to the volume of the solution. Allow to stand 12 hours. Filter, ignite, weigh as strontium sulphate, and calculate to strontium. Test spectroscopically for absence of calcium. Subtract from the total oxids of strontium and calcium the strontium oxid equivalent to the strontium sulphate found. The difference is calcium oxid. Calculate to calcium. As a check on the calcium oxid, evaporate to dryness the filtrate from the strontium nitrate, dissolve the calcium nitrate in water, precipitate as oxalate, filter, wash, ignite, and weigh as calcium oxid.

(*g*) *Magnesium.*

Concentrate solution C to about 200 cc, add from 2 to 3 grams of di-ammonium hydrogen phosphate[2] and sufficient hydrochloric acid to clear the solution when the ammonium phosphate is all dissolved; when cold make slightly alkaline with ammonium hydroxid, constantly stirring. Add 1 to 2 cc excess of ammonium hydroxid. Allow to stand overnight. Filter off the supernatant liquid and wash three or four times by decantation with 2.5 per cent of ammonium hydroxid. Dissolve the precipitate in hydrochloric acid. Dilute to about 150 cc. Add a little di-ammonium hydrogen phosphate and precipitate with ammonium hydroxid as before. Allow to stand overnight, filter, wash free from chlorids, ignite, blast, and weigh as magnesium pyrophosphate; calculate to magnesium.

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[1] Usually two or three washings are sufficient. Owing to the solubility of strontium oxalate excessive washing gives low results.

[2] Disodium hydrogen phosphate or sodium ammonium hydrogen phosphate may be used.

4. SULPHURIC ACID, SODIUM, POTASSIUM, LITHIUM.

(a) *Sulphuric acid.*

A preliminary examination should be made, using from 100 cc to 250 cc of the water to determine the quantity of sulphates. The alkali salts present can be approximated by calculating the amount of sodium necessary to combine with the excess of acids (chlorin, sulphuric, and bicarbonic acid) over the calcium and magnesium.

Evaporate such a quantity of the water that the weight of barium sulphate will not exceed 1 gram and the weight of the mixed chlorids will not exceed 0.5 gram. Acidify the quantity of water (usually from 1 to 5 liters) with hydrochloric acid. Evaporate to dryness in a platinum dish and remove silica by two evaporations as under 3 (*a*), page 77, using not more than 2 cc of hydrochloric acid for the final solution. Combine filtrate and washings from the silica determination. Concentrate to about 150 to 200 cc. Heat to boiling and precipitate with slight excess of 10 per cent barium chlorid, added very slowly, drop by drop, constantly stirring. Cover and allow to stand on the steam bath overnight. Filter, thoroughly wash the precipitate of barium sulphate with hot water, dry, ignite over Bunsen burner, and weigh. Calculate to sulphate (SO_4). The filtrate is solution E.

(b) *Sodium, potassium, lithium.*

Evaporate solution E to dryness in a platinum dish and ignite the residue to a very low redness to remove all traces of ammonium salts. Dissolve the residue in the dish with about 200 cc of water and precipitate with milk of lime or a solution of barium hydroxid. Boil, allow to stand half an hour, and filter off the insoluble magnesium hydroxid. Thoroughly wash the precipitate with hot water and combine the filtrate and washings. If the precipitate of magnesium is large, it is advisable to dissolve in a small amount of hydrochloric acid, evaporate to dryness, take up with water, and again precipitate with milk of lime or barium hydroxid as before. Concentrate the two filtrates and washings to about 200 or 250 cc. Add ammonium hydroxid and a sufficient quantity of ammonium carbonate to precipitate calcium and barium. Allow to stand on a steam bath for from one to two hours. Filter off the supernatant liquid, dissolve in hydrochloric acid, and precipitate as before and thoroughly wash with hot water. Evaporate filtrate and washings to dryness, dry and drive off the ammonium salts by gentle heat. Take up the residue with water; filter through a small filter, using as little wash water as possible; evaporate to a small volume and again precipitate with a drop or two of ammonium hydroxid and two or three drops of ammonium carbonate and oxalate. If any precipitate appears (which is not usually the case), filter and repeat the process. Evaporate the filtrate to dryness and drive off all ammonium salts by heating in platinum to a little below redness. Take up the residue with a little water, filter into a small platinum dish, add a few drops of hydrochloric acid, and evaporate to dryness. Dry in an oven, heat to a little below redness, cool in a desiccator, and weigh the combined chlorids of potassium, sodium, and lithium. Repeat the heating to constant weight. Dissolve the mixed chlorids with hot water, filter, and wash. Return the filter paper and small amount of residue usually found to the dish, dry, ignite, and weigh. The difference between the original weight of mixed chlorids and dish, and the weight of dish and small amount of residue equals the mixed chlorids. The combined chlorids are transferred to a small Erlenmeyer flask (50 or 100 cc capacity) and the solution evaporated nearly, but not quite, to dryness. Add about 30 cc of redistilled amyl alcohol. Connect flask, stopper of which carries a thermometer, with a condenser[1] and boil until the temperature rises approximately to the boiling point of amyl alcohol (130° C.), showing that all the water has been driven off. Cool slightly and add a drop of hydrochloric acid to convert small amounts of lithium hydroxid to lithium chlorid. Connect with the condenser and continue the boiling to drive off again all water and until the temperature reaches the boiling point of amyl alcohol. The content of the flask at this time is usually from 15 to 20 cc. Filter through a small paper or a

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[1] The amyl alcohol may be boiled off without the use of a condenser, but the vapors are very disagreeable.

Gooch crucible into a graduated cylinder and note exact quantity of filtrate which determines the subsequent correction. Wash the precipitate with small quantities of dehydrated amyl alcohol. Evaporate the filtrate and washings in a platinum dish to dryness on the steam bath, dissolve the residue in water, and add a few drops of sulphuric acid. Evaporate on a steam bath and expel the excess of sulphuric acid by gentle heat over a flame. Repeat until carbonaceous matter is completely burned off. Cool and weigh the dish and contents. Dissolve in a small quantity of hot water, filter through a small filter, wash and return filter to dish; ignite and weigh. The difference between the original weight of dish and contents and the weight of the dish and small amount of residue equals the weight of impure lithium sulphate.[1] From this weight subtract 0.00113 gram for every 10 cc of amyl alcohol filtrate[2] on account of the solubility of sodium and potassium chlorids in amyl alcohol. Calculate lithium from the corrected weight of lithium sulphate. Dissolve the mixed chlorids from flask and filter with hot water, evaporate to dryness, ignite gently to remove amyl alcohol, filter and thoroughly wash; concentrate the filtrates and washings to from 25 to 50 cc. Transfer to a porcelain dish and add platinic chlorid solution sufficient to convert sodium and potassium to platinic chlorids and evaporate to dryness. Treat the residue with 80 per cent alcohol and filter and continue washing until the excess of platinum chlorid and sodium platinic chlorid has been removed.

Dry the filter, dissolve the residue with hot water, and transfer to a weighed platinum dish. Evaporate on the steam bath, dry in the oven at 100° C. for 30 minutes and weigh as potassium platinic chlorid: calculate to potassium chlorid. To the weight of potassium chlorid add 0.00051 gram for every 10 cc of amyl alcohol used in the extraction of the lithium chlorid, which corrects for the solubility of the potassium chlorid in amyl alcohol. Calculate to potassium.

The weight of sodium chlorid is found by subtracting the combined weights of lithium chlorid and potassium chlorid (corrected) from the total weight of the three chlorids. Calculate sodium chlorid to sodium.

5. PHOSPHORIC ACID.

[As in Circular 52, p. 8.]

6. CHLORIN.[3]

To 100 cc of the water add a few drops of phenolphthalein. If a red color is shown titrate the carbonates thus indicated to bicarbonates with tenth or twentieth-normal sulphuric acid. (Add at the rate of a drop every few seconds until the red color disappears.) Add 1 cc of potassium chromate solution[4] (in the beginning if a red color is not shown with phenolphthalein) and titrate the chlorids with a standard solution of silver nitrate (1 cc=1 mg of chlorin). If iodids and bromids are found make the equivalent correction. If sufficient chlorids are present to consume more than 25 cc of the standard silver nitrate for 100 cc of the water, they should be determined by precipitating in nitric acid solution and weighing the silver chlorid found.

7. NITRIC ACID.[5]

Transfer to a porcelain dish 100 cc of the water or if the nitrates are high, such a volume as will contain about 1 mg of nitrogen. Add sufficient twentieth-normal sulphuric acid to nearly but not quite neutralize all the alkalinity. Add standard silver sulphate free from nitrate (4.3969 grams per liter, 1 cc equals 1 cc of standard silver nitrate solution equals 1 mg of chlorin per cubic centimeter) precipitating all but about 0.5 mg of the chlorin. Heat to boiling, allow to settle, or add a little aluminum cream, filter and wash with small amounts of hot water. Evaporate the filtrate to dryness in porcelain; when cold add 2 cc of disulphonic acid[1] reagent, rubbing with a glass rod to insure intimate contact. Dilute with distilled water and add slowly ammonium hydroxid until the maximum color is developed. Transfer to a colorimetric cylinder, filtering if necessary, and compare with a standard potassium nitrate solution (containing 0.01 mg nitrate in 1 cc) which has been treated in like manner with phenol disulphonic acid reagent. Compare in the usual manner and record as nitrogen in the form of nitrates.

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[1] The purity of the lithium sulphate should be tested by adding small amounts of ammonium phosphate and ammonium hydroxid, which will precipitate any magnesium present with the lithium sulphate. Any precipitate appearing after standing overnight should be collected on a small filter and weighed as magnesium pyrophosphate, calculated to sulphate and subtracted from the weight of impure lithium sulphate.

[2] Exclusive of the amyl alcohol used in washing residue because of the slight solubility of solid mixed chlorids in amyl alcohol.

[3] Substitute for chlorin method given in Cir. 52, p. 6.

[4] Dissolve 5 grams of potassium chromate in 100 cc of distilled water, add a solution of silver nitrate till a slight permanent red precipitate is produced. After standing several days siphon off the clear solution.

[5] Substitute for the method given in Cir. 52, p. 4.

8. FREE AND ALBUMINOID AMMONIA.[2]

Connect a large flask of about 1.5 liters capacity with an upright bulb condenser by means of a rather large glass tube and a soft rubber stopper or a recently extracted cork stopper. Place in the flask 5 cc of a concentrated solution of sodium carbonate and 500 cc of ammonia-free water. Distil off in 50-cc Nessler jars until no further traces of ammonia are obtained, and continue the distillation until the solution in the flask has been reduced to about 200 cc. Cool slightly, add 500 cc of the water under examination, and distil[3] into 50 cc Nessler jars until ammonia ceases to be given off. Four or five jars are usually sufficient. Nesslerize and compare the depth of color with other jars containing known amounts of standard ammonium chlorid solution (0.01 mg of ammonia (NH_3) in 1 cc) made up to 50 cc with ammonia-free water and Nesslerized in a similar manner. Report as ammonia (NH_3). Cool the flask and add 50 cc of recently boiled permanganate solution (prepared by dissolving 200 grams potassium hydroxid and 8 grams of potassium permanganate in 1 liter). Distil[3] off into 50-cc Nessler jars until ammonia ceases to come off. Nesslerize and compare in a similar manner as for the determination of free ammonia.

9. BROMIN, IODIN, ARSENIC, AND BORON.

[Circular 52, pp. 9, 10, and 11, unchanged.]

10. CARBONIC (CO_3) AND BICARBONIC (HCO_3) ACIDS.

To 100 cc of the water add a few drops of phenolphthalein and if a pink color is produced titrate with twentieth-normal acid potassium sulphate or sulphuric acid, adding a drop every two or three seconds, until the red color has disappeared. Multiply by the factor 0.003, which gives the grams of carbonic acid in 100 cc. To the colorless solution from this titration or to the original solution if no color is produced with phenolphthalein, add one or two drops of methyl orange and continue the titration without refilling the burette and note the total reading. If carbonic acid is absent, multiply the total burette reading by the factor 0.00305, which gives the value of bicarbonic acid in grams per 100 cc. If carbonic acid is present, multiply the reading with phenolphthalein by 2 and subtract from the total reading of the burette. Multiply the difference by the factor 0.00305, which gives the bicarbonic acid in grams per 100 cc. Multiply the result by 10 to convert to grams per liter.

11. FREE CARBON DIOXID.

[As in Circular 52, p. 11.]

12. METHOD OF REPORTING RESULTS.

[As in Circular 52, p. 12.]

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[1] Prepared by dissolving 25 grams of pure white phenol in 150 cc of chemically pure sulphuric acid, concentrated, and 75 cc of fuming sulphuric acid (13 per cent SO_3) and heating at 100° C. for two hours.

[2] Substitute for methods for free and total ammonia, Cir. 52, p. 5.

[3] At the rate of one tube full in 15 minutes.

REPORT OF COMMITTEE A ON RECOMMENDATIONS OF REFEREES.

By J. P. STREET, *Chairman.*

(Nitrogen, potash, phosphoric acid, soils, inorganic plant constituents, insecticides, and water.)

PHOSPHORIC ACID.

It is recommended—

(1) That the following method of analysis [Wagner method, see p. 12] be adopted provisionally pending further study and the gathering of more complete data in regard to field and pot experiments.

Adopted.

(2) That further work be done with the citrate of ammonia magnesia mixture method [see p. 12] and the official volumetric method, using the Wagner method of making the citric solution of the slag, as given under recommendation 1.

Adopted.

(3) That the referee on phosphoric acid for 1912 study the conductivity method for making the neutral ammonium citrate solution. (See J. Amer. Chem. Soc., 1911, 33: 711; J. Ind. Eng. Chem., 1911, 3: 559.)

Adopted.

NITROGEN.

It is recommended—

(1) That the study of methods for the determination of available nitrogen be continued, and that the alkaline permanganate and neutral permanganate methods as now modified be studied as applied to crude stock and to commercial fertilizers.

Adopted.

(2) That the recommendation made on page 129 of Bulletin 116[1] be studied with a view to its applicability to complete fertilizers and meat products.

Adopted.

This recommendation reads as follows: "(5) That on page 7 of Bulletin 107, after insert recommended under (4), add the following: "Approximately 0.7 gram of mercuric oxid, or its equivalent in metallic mercury, may also be added, before the addition of the potassium sulphate, but if mercury be used potassium sulphid must be employed, as in the Kjeldahl method, in the distillation."

(3) Mr. E. L. Baker's resolution to the effect that Salle's method for the determination of nitrogen in commercial nitrates be referred to the referee for 1912 for trial was adopted. The method is given on page 28.

SOILS.

It is recommended—

(1) That the methods approved and referred to the association for final action in 1910, under recommendations 1, 2, and 3, page 3 of Circular 52, be finally adopted as official.

Adopted.

These recommendations read as follows:

(1) That the modified J. L. Smith method for total potassium be made an official method of this association, and that the clauses "and transfer to a filter" and "after washing free of chlorids" in this method be replaced by the following sentence: "After washing four or five times by decantation with hot water, throw on the filter and wash well, 250 to 300 cc of wash water being sufficient."

(2) That the magnesium nitrate method for total phosphorus be made an official method of this association.

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[1] All bulletin references are to Bureau of Chemistry series, United States Department of Agriculture.

(3) That the sodium peroxid fusion method for total phosphorus be made an official method.

(2) That the referee on soils continue his research for a method or methods that will permit of an accurate estimation of the lime requirements of soils.

Adopted.

(3) That the Rather method (p. 52, see also J. Ind. Eng. Chem., 1911, 3: 660) for humus be further studied with a view to substituting it for the present official method.

Adopted.

(4) That the modified volumetric cobalti-nitrite method be further studied in its application to soils (J. Ind. Eng. Chem., 1909, 1: 302; Bul. 137, p. 25).

Adopted.

POTASH.

It is recommended—

(1) That the Drushel volumetric cobalti-nitrite method be further studied in its application to mixed fertilizers (Bul. 132, p. 21).

Adopted.

(2) That a further study of the gravimetric cobalti-nitrite method be made in the case of mixed fertilizers, using the modification suggested by Itano of precipitating the phosphates with milk of lime before adding the cobalti-nitrite reagent.

Adopted.

(3) That the proposed modification of the official method (p. 29) be approved and be referred to the association for final action in 1912.

Adopted.

(4) That the factors for calculating potash from potassium platinic chlorid (Bul. 107, Rev., p. 12) be revised to read as follows:

Under "(c) Factors," read "For the conversion of potassium platinic chlorid to potassium chlorid, use the factor 0.3067; to potassium sulphate, 0.3585; to potassium oxid, 0.1938." (This change is made in accordance with the new atomic weights.)

Adopted.

INORGANIC PLANT CONSTITUENTS.

It is recommended—

(1) That the molybdate method in the form in which it is now presented for the separation of ferric and aluminic oxids in an ash solution be approved, with a view to its adoption as an official method in 1912. [See p. 61.]

Adopted.

(2) That the oxalate method for the separation of ferric and aluminic oxids in an ash solution be further studied on a synthetic solution, with a view to its final adoption by the association. [See p. 61.]

Adopted.

(3) That cooperative work be done on the molybdate method as extended and described in the 1911 report for the separation of calcium and magnesium in an ash solution. [See p. 65.]

Adopted.

(4) That cooperative work be done on the method entitled "The determination of total sulphur in organic matter," described by Herman Schreiber in Circular 56.

Adopted.

WATER.

It is recommended—

(1) That the methods as proposed for the examination of waters (see Cir. 52, pp. 4 to 14) be accepted as the provisional methods of the association.

Adopted.

INSECTICIDES.

It is recommended—

(1) That the chromate method (Method II) for total lead oxid in lead arsenate be adopted as official (Bul. 137, p. 41, and Cir. 66, p. 2).

Recommendation adopted and referred to association for final action in 1912.

(2) That the method for water-soluble arsenic oxid in lead arsenate be further studied as regards time of standing for the solution of soluble arsenic, and the effect of different temperatures thereon (Bul. 107, Rev., p. 240).

Adopted.

(3) That the method for total sulphur in lime-sulphur solutions (Bul. 107, Rev., p. 34) be changed as follows: Under "2. Determination," line 1, after "measure" insert "and accurately weigh"; after "sample" strike out "in" and insert a comma and the words "transfer to"; and that the method as thus changed be recommended for final adoption as official in 1912.

Adopted.

(4) That the gravimetric method for sulphur as sulphids and polysulphids in lime-sulphur solutions, as given in the referee's report [p. 70], be adopted as official.

Recommendation adopted and referred to the association for final action in 1912.

(5) That the volumetric method for sulphur occurring as thiosulphate in lime-sulphur solutions, as given in the referee's report [p. 70], be adopted as official.

Recommendation adopted and referred to the association for final action in 1912.

(6) That the method given [by the referee] for sulphur occurring as sulphates and sulphids in lime-sulphur solutions [p. 70] be changed to read as follows and as changed be adopted as official:

Sulphur as sulphate and sulphite.—To the solution from the determination of thiosulphate add 2 or 3 drops of hydrochloric acid, warm on steam bath, precipitate with barium chlorid solution, stirring vigorously for several minutes; let stand in the cold overnight, filter, and obtain weight of barium sulphate. From this weight calculate sulphur.

Recommendation adopted and referred to the association for final action in 1912.

The following recommendations, which were adopted by the association in 1909, are hereby renewed for final action:

(7) That "Method II" for total arsenious oxid in London purple, as given in Bulletin 107, Revised, page 29, be dropped from the methods of analysis.

Adopted.

(8) That "Method II" for total arsenic oxid in London purple, as given in Bulletin 107, Revised, page 29, be dropped from the methods of analysis.

Adopted.

The three following recommendations, adopted by the association in 1910, are hereby renewed for final action:

(9) That "Method I" for total arsenious oxid in London purple, as given in Bulletin 107, Revised, page 28, be adopted as official.

Adopted as official.

(10) That "Method III" as proposed by the referee in 1909 (Bul. 132, p. 43) for total arsenic oxid in London purple be adopted as official and designated as "Method II."

Adopted as official.

(11) That the Gatehouse method (Sutton's Volumetric Analysis, 9th ed., p. 201; Cir. 10, Rev., p. 6) for the determination of chlorin in cyanids be adopted as official.

Adopted as official.

(12) That the provisional methods for the analysis of lead arsenate (Bul. 107, Rev., p. 239) be changed in accordance with recommendation 7 of the referee in 1910 (Bul. 137, p. 47), and, as changed, adopted as official.

Recommendation adopted and referred to the association for final action in 1912.

SEPARATION OF NITROGENOUS BODIES (MILK AND CHEESE).

It is recommended—

(1) That the Folin method[1] for the determination of ammonia be further studied. (Zts. physiol. Chem., 1902-3, 37:161.)

Adopted.

(2) That the Van Slyke method for the determination of amino acid nitrogen be further studied. (D. D. van Slyke, Ber. d. chem. Ges., 1910, 43 (3):3170.)

Adopted.

REPORT OF COMMITTEE ON AMENDMENTS TO THE CONSTITUTION AND BY-LAWS.

By L. L. VAN SLYKE, *Chairman.*

(1) The following resolution, introduced by W. A. Withers, was favorably reported by the committee and was adopted by the association:

Resolved, That compliance with section 4 of the constitution necessitates the appointment of referees and associate referees by the retiring, rather than by the incoming, executive committee: *Resolved further,* That the secretary of the association is hereby requested to provide a place on the program for the announcement of the appointment of referees and associate referees.

(2) The following resolutions were introduced by O. M. Shedd and referred to the committee, with the results stated:

(*a*) *Resolved,* That section 4 of the constitution of this association be changed to read as follows:

There shall be appointed by the executive committee at the regular annual meeting from among the members of the association a referee, and, after consultation with him, such associate referees for each of the subjects to be considered by the association as that committee may deem appropriate.

The committee reported unfavorably on the adoption of this resolution as an amendment to the constitution, but moved that a standing rule be adopted to the effect that the executive committee consult with each referee in the appointment of associate referees.

The report of the committee was adopted.

(*b*) *Resolved,* That a new section be added to the by-laws to follow section 4, and to read as follows:

As soon as possible after the annual meeting of the association each retiring referee shall transmit a copy of his report and recommendations, together with a statement of the action taken by the association upon the same, to the referee for the next year.

The committee reported this amendment favorably as an addition to section 4, and the report was adopted:

(*c*) *Resolved,* That paragraph 3 of the by-laws be amended by adding the following words:

That the referee on each subject be ex officio member of the subcommittee on recommendations of referees which passes upon his recommendations, but only for the purpose of such action as relates to his own work.

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[1] See referee's report, p. 185, for recent modification by Folin.

The committee reported the resolution unfavorably, but stated that it was the sense of the committee that, except when impossible, opportunity should be given each referee for full consultation with the committee on recommendations before final action by the committee on the recommendations of the referee.

The report of the committee was adopted.

Brief verbal reports were made by the chairmen of the following committees:

Committee on appropriation.—Mr. Street, as chairman, reported that no meeting had been held, and since the Proceedings were to be published by the Department of Agriculture and the dues now paid by the members covered all other expenses he asked that the committee be discharged. After some discussion, however, the report of the committee was accepted by the association on a motion by Mr. Frear, but the committee was continued.

Committee on food standards.—Mr. Frear, as chairman, made a report of progress, stating that the committee had held a meeting in August in Duluth at which a special line of work was taken up and would be pushed as rapidly as possible.

Committee on participation in the Eighth International Congress of Applied Chemistry.—Mr. Street, as chairman, stated that he thought that the committee no longer had any function to perform, inasmuch as the ground was well covered by Mr. Cameron's and Mr. Bigelow's committees. It was urged that the members of the association cooperate with the various sections and insure that the association be adequately represented on the program of the congress. The effect of the ruling that no papers presented at the congress could be published elsewhere was also mentioned, and attention called to the fact that in the preliminary announcement of the congress the association had not been included. The chairman asked that the committee be discharged, and upon motion the report was accepted and the committee discontinued.

Committee on the testing of chemical reagents.—Mr. Kebler, as chairman, made the following brief report of progress: At the last meeting the committee reported on the desirability of using certain designations in connection with chemical reagents. These recommendations were also forwarded to the chairman of a similar committee of the American Chemical Society, but they have not been discussed as fully as the committee hoped they might be.

It is fairly well established that the designation "C. P." serves no good purpose except in a few special instances. The result is that most manufacturers are rapidly eliminating it from their price lists; in fact, some have done so completely. It was intended to present at this meeting the specifications referred to in the recommendations last year for various chemical reagents with the possibility of some action. Unfortunately this was impossible, but it is believed that the specifications can be presented at the next meeting. It is urged that the subject of chemical reagents be given more consideration by the association in the future than has been the case in the past.

The report of the committee was received.

On motion by Mr. Frear the association authorized the secretary-treasurer to pay the balance of a bill owing by the food standards committee for printing, amounting to $31.03.

The association adjourned.

SECOND DAY.

TUESDAY—MORNING SESSION.

REPORT ON FOOD ADULTERATION.

By A. S. MITCHELL, *Referee.*

The past year has been an active one both in the perfecting of new methods and in the collaborative trying out of the present standards. I shall leave the details of the work for discussion by the associates who have so ably and disinterestedly accomplished it, and will but touch upon such points as seem of interest to all.

The referee on cereals has made a good beginning on methods for the analysis of wheat flour, but we ask only for the adoption of such portions as seem most free from criticism.

The referee on vinegar recommends a partial revision of methods. The Ross method is offered for glycerin as an index of purity of cider vinegar. This is novel, but when it is noted that glycerin is a congener of alcoholic fermentation and is separated from low wines in their distillation, while it remains in the final product in undistilled vinegars, its possibilities are apparent.

The referee on fats and oils has conducted a study on beef fat in lard. Tests by the Belfield method proved less satisfactory than by the Emery method, a method based on the melting point of the crystallized glycerids.

Collaborative work on the methods for the separation and identification of artificial colors has shown a reliability almost surprising, considering the complexity of the subject.

Extensive work has also been done on the flavoring extract methods. Improvements in methods for vanilla extract are reported which result in saving time and increasing accuracy. The unreliability of certain other methods has been demonstrated.

Under spices, the study of paprika has brought out a new characteristic, the remarkably high index of refraction of its oil. The collaborative work on the crude fiber method as applied to prepared mustard indicates the reliability of the present method.

A large amount of work has been done on ketchups. Three collaborators have made lengthy analyses involving 36 determinations on each of the three samples sent out. It will be noted that food chemists are looking less upon the wine when it is of varied hue and are more closely scrutinizing the tomato when it is overripe. This study has emphasized the work of Bacon and Dunbar as to the value of lactic and citric acid ratios as an indication of decay in tomato products. Citric acid, which is present in sound, ripe tomatoes, rapidly disappears upon decomposition, with a corresponding development of lactic acid. The work done upon the methods offered shows the need of their further study.

The referee on dairy products reports a method for obtaining fat from ice cream and similar foods in sufficient quantity to admit of its thorough examination.

Progress is also reported in the examination of methods for the determination of caffein in tea and coffee. The question of coloring matter in tea came up too late for action this year. For the coming year, it is suggested that the referee make this a special study. A draft of methods has been issued by the United States Treasury Department, but more searching microscopical methods seem desirable.

A method is needed for the determination of diastase in fermented liquors for which diastatic action is claimed. Standard methods are also needed for the examination of beverages, particularly so-called soft drinks, and for the examination of coatings and glazings in confectionery.

The desirability of the appointment of a referee on methods for the determination of arsenic, lead, tin, copper, barium, and the heavy metals is obvious.

Microscopical methods are gaining prominence in the examination of prepared foods, spices, coloring of tea, etc., and their development would seem to warrant the appointment of a special referee.

The work of the food analyst is increasing in complexity and ramifying in new directions. This calls for an increased loyalty and sacrifice of time from the food chemists of this association, to enable us to take up the varied, and, too frequently, lengthy, methods and try them out, ever keeping in mind accuracy, simplicity, and speed. In concluding, I wish to thank my associates for the interest they have shown and the time and attention which they have given to this work.

REPORT ON SPICES.

By R. W. Hilts, *Associate Referee.*

The work done this year falls under the two headings of ether extract of paprika and crude fiber in prepared mustard.

Paprika Extract.

In accordance with last year's recommendations, the associate referee has made some further study of the ether extract of paprika and of the method of drying it. No samples were sent out, as the nature of the work rendered it unsuitable for cooperative investigation. The work was divided into two parts:

(A) Some experiments were made on the drying of ether extract of paprika under the conditions of the provisional method, last year adopted, as compared with drying for the same periods in a current of dry hydrogen. For this purpose a sample of Hungarian paprika, which appeared entirely normal, was dried over sulphuric acid and extracted with pure dry ether. The ether solution was filtered and four equal portions of it measured into glass-stoppered flasks and the ether distilled off as usual. All four flasks were dried side by side in the same water oven for two successive half-hour periods. Two were dried in air, as in the provisional method, but the other two were fitted with a suitable arrangement of two-hole stoppers and glass tubes by means of which a rapid current of hydrogen, dried by sulphuric acid, was circulated through them. This passed in at the top and out at the bottom through the longer tube, thus assuring rapid drying and displacement of the ether vapor. The results are as thus tabulated:

Comparison of drying paprika extract in air and in hydrogen.

Time.	Weight—			
	Drying in air.		Drying in hydrogen.	
	Gram.	*Gram.*	*Gram.*	*Gram.*
After one-half hour	0.3024	0.3055	0.3038	0.3046
After 1 hour	.3021	.3053	.3034	.3043

The data indicate that drying proceeded with equal speed under both conditions and that the weight changes, though slight, were practically identical. The iodin numbers also agree very well indeed, 133.3 and 132.7 on the samples dried in air, and 133.9 and 133.1 for those dried in hydrogen. The values of the two methods overlap, and the extreme differences are insignificant, as they are well within the range of experimental error. Hence it does not appear that the ether extract of paprika is appreciably oxidized by one hour's heating in air in the water oven under the conditions of the provisional method. Excessively long heating will undoubtedly change the extract and give lower iodin numbers, as shown by Seeker[1] and Lowenstein and Dunne,[2] but such heating is unnecessary, using pure, dry, freshly distilled ether. The referee has never found more than an hour's heating necessary. There does not seem, then, any reason for supplanting the present simple method by anything more cumbersome.

(B) For the purpose of making a further study of paprika extract, with a view to detecting foreign oils other than olive, a considerable quantity of the oil was prepared. By courtesy of the Bureau of Chemistry, the referee was furnished with a large sample of whole Spanish paprika, or pimenton. The fruit was whole, unbroken, and in good condition. The pods were globular, about 2 inches in diameter, and were strung on strings. The stems were separated and rejected and the sample ground in the laboratory and examined by the methods officially adopted. The composition of the sample and the results were as follows:

Stems	108.5 grams (8.89 per cent)
Seeds and placentæ	429.0 grams (35.17 per cent)
Shells	682.5 grams (55.94 per cent)
Analytical determinations:	
Ether extract (per cent)	11.40
Iodin number	136.0
Total ash (per cent)	7.05
Ash insoluble in acid (per cent)	0.07

From the data available the values appear to be normal. After rejection of the stems the sample contained 38.6 per cent of seeds and placentæ and 61.4 per cent of shells. It had a good red color after grinding.

Eight hundred grams of the material were packed in a percolator and extracted by slow percolation with redistilled petroleum ether boiling below

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[1] U. S. Dept. Agr., Bureau of Chemistry Bul. 137, p. 82.

[2] J. Ind. Eng. Chem., 1910, 2: 139.

55° C.[1] Percolation was continued until most of the oil was removed. The oil was freed from the last of the petroleum ether by heating in a flask immersed in boiling water, under a partial vacuum, meanwhile admitting a slow current of dry hydrogen to displace the ether and prevent oxidation. The oil was finally filtered at a moderate temperature. The yield was about 75 grams or 9.4 per cent. The extract was a rather viscous oil, fluid at all ordinary temperatures and of a very intense red color, almost black in bulk, and with an odor suggestive of the spice. It was examined with the following results:

Specific gravity 15.6°/15.6° C	0.9322
Index of refraction at 40° C	1.4858
Index of refraction at 15.6° C	1.4953
Iodin number	137.9
Reichert-Meissl number	0.82
Solid fatty acids (per cent)	10.83
Acetyl value (filtration method)	21.0
Acetyl value (corrected for soluble acids)	9.45

The absorption spectrum, in ether solution, possessed no bands and showed nothing distinctive. The intense color of the oil interferes so much with the indicator that the referee has not as yet succeeded in determining the saponification number. Ordinary methods of bleaching were only partly successful, and attempts to use an external indicator were unsatisfactory. It seems that any method of bleaching sufficiently rigorous to be successful might materially alter the oil.

The iodin number of the oil thus prepared agrees well with that found in the preliminary examination by the provisional method, pointing to the similarity of the oil to that extracted by ethyl ether. It is probably purer than an ether extract would be. Contrary to the figures of Szigeti,[2] published by the associate referee last year, neither the acetyl value nor the Reichert-Meissl number appears to be higher than those of the ordinary oils and fats. Strict comparison with his results is of course impossible, since his method of preparing the extract is unknown. Partial oxidation and hydrolysis might account for his low iodin numbers and high acetyl values. The index of refraction, however, is remarkably high, even above Szigeti's values, which were 1.489–1.490 at 15° C. Of all the constants determined the index of refraction is the only one, aside from the iodin number, which seems to be promising for the detection of foreign oils. It has also the advantage of requiring little material. The determination of this constant on numerous samples is accordingly desirable in order to observe its range of variations and thus to test further its value as a criterion of purity.

CRUDE FIBER IN PREPARED MUSTARD.

Last year's recommendation for the comparative study of the determination of crude fiber, with and without preliminary drying and extraction of fat, was made with special reference to prepared mustard. This year's referee on condiments other than spices kindly waived any interest he might have, and accordingly a large sample of prepared mustard was procured from a reputable grinder who stated that it was ground from the whole seed only. It was mixed with particular care, and eight samples were sent out with the following instructions. Method II, with preliminary drying and fat extraction, was suggested by Mr. L. M. Tolman as having been tried in the Washington Food Inspection Laboratory. Method III, involving previous removal of the fat by washing with alcohol and ether without drying, was suggested by the associate referee.

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[1] Petroleum ether was chosen in preference to ethyl ether because of the practical difficulties of drying the large mass of material and keeping it dry, if dry ether was to be used. A preliminary experiment indicated that the iodin numbers of oils extracted by both solvents are practically the same and that hence the oils must be of nearly the same composition.

[2] U. S. Dept. Agr., Bureau of Chemistry Bul. 137, p. 85.

INSTRUCTIONS TO COLLABORATORS.

Determine crude fiber by each of the three methods below. Also determine moisture in the original sample by the official method, and also moisture in the dried, and the ground air-dry sample in Method II. Determine ether extract in the air-dry sample in Method II, this being incidental to the crude fiber determination, and, for comparison, determine ether extract by the official method (I). The moisture determinations are indispensable. Use particular care in sampling. Transfer to a larger receptacle if necessary, and mix thoroughly with a spoon before removing each portion for analysis. Report all actual results obtained on the fresh and air-dry sample and also report crude fiber and ether extract calculated to dry substance.

Method I (*official*).

(Bul. 107, Rev., pp. 167–168; Proceedings A. O. A. C., 1905, Bul. 99, p. 73.)

Solids.—Dry 5 grams in a flat-bottomed platinum dish on a water bath until the mixture appears dry, and finally dry to constant weight at 100° C. in a water oven. First distribute the sample evenly over the bottom of the dish with a little water.

Crude fiber.—Follow the official method as outlined (Bul. 107, Rev., p. 168), remembering the additional directions last year adopted: "The fiber, however, should finally be washed successively with alcohol and ether until all fat is removed." Be particularly careful to break up thoroughly all lumps by shaking as directed. Stopper the flask and shake vigorously if necessary. The first filtrations may be made on paper or other convenient filter. A circle of closely woven linen in a 4-inch Buechner funnel with light suction gives a very rapid and satisfactory filtration. The linen circle is then spread out on the side of a short-stem 6-inch funnel, and the fiber washed back into the flask by means of a policeman and a wash bottle containing the hot 1.25 per cent alkali. The last filtration should be made on paper as officially directed. For this an 11 cm S & S blue ribbon paper is convenient and rapid. All weighings of paper and crude fiber must be made in weighing bottles.

Ether extract.—Follow the official method. Use a lead bottle cap or a Hoffmeister schaelschen for drying the sample and finish the drying in the hot-water oven.

Method II.

Preparation of sample.—In an 8-inch porcelain evaporating dish spread out 100 grams of the sample and dry down on the steam or water bath. Finally dry in a vacuum oven, or if this is not available, dry for 3 to 4 hours more in a water oven. Let stand in the air for some hours to become air-dry; then scrape out of the dish and grind in a small drug mill to pass a 40-mesh sieve.

Moisture in air-dry sample.—Dry about 2 grams in a flat-bottomed dish in the water oven.

Ether extract.—Weigh out 2 grams of the air-dry sample, inclose in a folded 11 cm hardened filter paper, dry somewhat and extract in a Knorr or other suitable extraction apparatus for 16 hours with anhydrous ether.

Crude fiber.—Use residue from the ether extraction, and proceed according to the official method (Bul. 107, Rev., pp. 56, 164). Observe the comments on filtration made above. Final washing with alcohol and ether is unnecessary and is to be omitted.

Method III.

Crude fiber.—Weigh out 8 or 10 grams in a small beaker and add strong alcohol to a volume of about 75 cc. Allow to stand 15 to 20 minutes with occasional stirring and then filter through an 11 cm S & S hardened filter paper, transferring completely to the filter. Wash on the paper two or three times with alcohol and then 6 times with ether or until all fat is removed. Allow to drain, but not to dry or cake, and then rinse into the 500 cc Erlenmeyer flask with a little of the cold 1.25 per cent acid. Stopper, shake, make up to 200 cc with boiling acid and bring to a boil and boil gently for 30 minutes. Foaming may occur until the ether is all expelled, hence the flask had best be simply covered with a watch glass, which permits blowing into it by means of a glass tube if the foam approaches the top. Continue the determination as under instructions for the official method, except that final washing with alcohol and ether is omitted.

The preliminary washing with alcohol and ether can also be conveniently performed by aid of the centrifuge, shaking the samples up in stoppered tubes 6 in. × 1 in., then centrifuging and decanting off through the hardened filters, to which the solid material is finally transferred by means of ether.

Five collaborators made reports. Their names are as follows, the numbers indicating their results in the tables: (1) M. Boyle, Washington, D. C.; (2) A. W. Hanson, Kansas City, Mo.; (3) C. W. Harrison, Washington, D. C.; (4) R. W. Hilts, Philadelphia, Pa.; (5) H. E. Sindall, Philadelphia, Pa.; (6) C. R. Smith, New York, N. Y.

Cooperative results on solids and ether extract.

Analyst.	Solids.		Ether extract.			
	Method I.	Method II.	Method I.		Method II.	
	Original.	Air-dry.	Original.	Dry basis.	Air-dry.	Dry basis.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
1	17.53	90.18	3.63	20.74	16.14	17.90
	17.47	90.12	3.64	20.80	16.07	17.83
2	17.83	93.44	3.0	16.80	15.21	16.27
	17.86	93.46	2.9	16.24	15.15	16.21
3	17.66	93.83	3.47	19.62	16.27	17.34
	17.71	93.83	3.42	19.34	16.16	17.22
4	17.87	94.44	2.92	16.34	16.48	17.45
	17.87	94.40	[1] 2.93	16.39	16.47	17.44
5	17.54	95.77	3.15	17.96	17.34	18.05
	17.54	96.32	3.05	17.39	17.46	18.18
6	17.33	92.66	3.11	17.80	16.51	17.82
	17.61	....	....	....	....	....
Average	17.65	....	3.20	18.13	....	17.43

[1] Three additional determinations gave 2.90 per cent, 2.94 per cent and 2.95 per cent of ether extract. Both Knorr and Soxhlet extractors used.

Cooperative results on crude fiber.

Analyst.	Method I.		Method II.		Method III.	
	Original.	Dry basis.	Air-dry.	Dry basis.	Original.	Dry basis.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
1	1.27	7.24	6.61	7.33	1.21	6.91
	1.24	7.13	6.74	7.47	1.19	6.80
	....	....	[1] 5.91	[1] 6.55	....	....
	....	....	[1] 5.93	[1] 6.58	....	....
2	1.19	6.67	6.8	7.27	1.38	7.73
	1.26	7.06	6.4	6.85	....	....
3	1.085	6.14	6.99	7.45	1.115	6.31
	1.035	5.85	7.03	7.49	1.117	6.32
4[2]	1.09	6.10	6.62	7.01	1.02	5.71
	1.06	5.94	6.66	7.05	0.97	5.43
	....	....	6.83	7.23	1.02	5.73
5	1.08	6.16	7.15	7.44	0.95	5.42
	1.02	5.81	7.13	7.42	0.93	5.30
6[3]	1.48	8.47	7.59	8.19	1.30	7.44
	1.54	8.81	....	....	1.34	7.67
Average	1.19	6.78	....	7.24	1.13	6.40
Maximum	1.54	8.81	....	8.79	1.38	7.73
Minimum	1.02	5.81	....	6.55	0.93	5.30

[1] Values indicated were obtained by making both filtrations on linen and finally washing into a gooch for drying and ashing.

[2] Also found 1.41 per cent of salt.

[3] Analyst 6 made all filtrations on linen, finally washing into a large platinum dish for drying and ashing.

COMMENTS BY THE COLLABORATORS.

Mr. Boyle finds filtration and drying on paper tedious and much prefers to make both filtrations on cloth, finally washing the fiber into a gooch for drying and ashing. The second set of results under Method II were thus obtained and are unaccountably lower than the others.

Mr. Harrison notes the fairly good agreement between Method I and III and the higher results by II. He sees no apparent benefit in the preliminary treatment with alcohol and ether in Method III except more rapid filtration from the acid, which, however, is offset by the troublesome frothing on first boiling. The final washing with alcohol and ether in the official method he considers a decided advantage as facilitating drying. He prefers Method I to either of the others as being easiest of manipulation, and believes it should give satisfactory and comparable results by different analysts, when followed as outlined. He adds, "I also find that the 11 cm S & S blue ribbon papers, on which you suggest that the filtration be made, lose on an average about 0.005 gram in weight when 200 cc of boiling 1.25 per cent alkali are filtered through them and then washed with hot water until free from alkali. This treatment was tried on four papers, and about the same loss in weight occurred in each case, which indicates that there is a slight error produced in the fiber determination by filtering through paper."

Mr. Sindall prefers Method I. He found difficulties in Method II in removing the hardened material from the dish and also by caking during grinding in the mill, due to the oil. Method III offered no difficulties.

Mr. Smith found filtration through paper extremely unsatisfactory. He made all filtrations on linen, finally washing the fiber into a large dish.

DISCUSSION OF RESULTS.

The agreement on solids in the original indicates that the material was well mixed and that sampling is not difficult. The vagaries of the ether extract results will not be discussed here except to suggest that physical condition and degree of dryness must have some influence on the extraction. On crude fiber the agreement between the analysts is not nearly so good as could be wished and shows that the personal factor is of more consequence than differences in the methods. This is evident from the fact that the individual analysts generally run consistently above or below the general average in all the methods and not in a single method. However, because of this consistency of results, the average for each method should show its relative tendency to give high or low values, although these averages can have little or no significance as to the true or probable value for the sample. In fact the lower values are doubtless nearer the actual truth, since all the difficulties inherent in the methods, such as the effect of the oil in preventing free attack by the reagents, or persistence of any oil in the fiber in Method I, or the hard nature of the residue, or difficulty in grinding sufficiently fine, or loss of oil on the utensils used in preparing the sample in Method II, would all tend to throw results high rather than low. It thus appears from the averages on the dry basis that Method II tends to give the highest and hence probably the most inaccurate results of the three methods. In the opinion of the referee this fact alone, besides the difficulties in grinding the hard and oily sample to uniform fineness and the labor involved, more than offsets the real advantage of a small factor in calculating to a basis for comparison, and makes further experiment with this method inadvisable.

It might be expected that Method III, by reason of previous removal of oil, and also good mechanical condition, would give the lowest results. Such, indeed, appears to be the case, but the average is only slightly below that of the official method. In fact the results of Method III seem hardly to justify the extra labor involved, and the official method is practically as good, besides being simpler.

Undoubtedly the greatest chances for error in the official method are failure to break up all lumps thoroughly and insufficient washing with water, alcohol, and ether in the last of the process. Altogether, the official method seems to be about the best at present available, but it would seem advisable to strengthen confidence in it by submission to collaborators of samples of prepared mustard of known composition, if possible.

Recommendations.

It is recommended—

(1) That further study be made of the ether extract of paprika, particularly of the index of refraction, with a view to detecting added foreign oils.

(2) That samples of prepared mustard of known composition be submitted to collaborators for the determination of crude fiber by the present official methods.

INDEX OF REFRACTION OF THE ETHER EXTRACT OF PAPRIKA AND PIMENTON.

By D. L. Weatherhead.

At the last meeting of the Association of Official Agricultural Chemists a new method of obtaining the ether extract of paprika was adopted provisionally, and it was suggested that it would be of interest to obtain data on the refractive index of this extract to see if it would be a useful factor in indicating the presence of added oil.

Following this suggestion some data of this nature were collected. No special time was given to the work, but it was done in connection with routine analyses on commercial samples examined at the New York Food and Drug Inspection Laboratory of the Bureau of Chemistry. It is believed, however, that this work throws some light upon the value of the refractive index of the ether extract in indicating the presence of added oil.

The extracts were prepared from the 150 cc of ether solution remaining in the 250-cc flask after the removal of 100 cc for the determination of the amount and iodin number of the extract. The ether solution was filtered into an Erlenmeyer flask and the ether distilled off as in the regular method. The extract was dried in a water oven for half an hour and the refractive index taken at 40° C. in an Abbe refractometer. In all operations the same method of handling and the same precautions were observed as are given for the ether extract in the accepted method. Since it is customary to recover the anhydrous ether from the 150 cc of solution remaining after removal of the portion used in determining the extract, very little extra work is required to obtain the refractive index in the regular course of analysis.

One special precaution seems necessary in reading the refractive index. The entire field is red, one portion being dark red and the balance light red. The border of the dark zone which must be brought on a line with the intersection of the cross hairs shows no dispersion, but upon moving the screw of the compensator attached to the Abbe refractometer the border of the dark zone is seen to move up or down according to the direction in which the compensator is rotated. Only the position and not the distinctness of the border seems to be altered in this way. In order to make the readings in a uniform and comparable way the compensator was always set to give the lowest reading.

In some cases the extracts were allowed to stand for some time before filtering from the residue and also before reading, but the results obtained in these cases do not indicate that the factor is appreciably influenced thereby. So, although the method outlined is considered preferable, some latitude may be permitted in the details of preparing the extract for the refractive index which could not be permitted in determining the amount and iodin number of the extract.

After taking the refractive index the extracts were set aside in loosely-stoppered flasks and the reading again taken one to two months later, the second reading being in almost all cases practically the same as the first. After standing for nine months under these conditions many of the extracts solidified and became colorless.

As a summary of the indications furnished by the refractive index the following values have been taken from the detailed data obtained:

Extreme and average data on refractive indices of ether extracts of pure and adulterated paprika and pimenton (reading at 40° C.).

Data.	Paprika.		Pimenton.	
	Pure.	With added oil.	Pure.	With added oil.
Minimum	1.4720	1.4640	1.4678	1.4660
Maximum	1.4825	1.4658	1.4705	[1] 1.4780
Average	1.4766	1.4649	1.4725	1.4696
Number of samples	25	2	[2] 16	10

[1] Other factors in the analysis indicate that very little oil could have been used in this sample.
[2] One sample repeated.

In general the refractive index varies less upon the addition of oil than do the ether extract and iodin number of the ether extract, but on the other hand, it also varies less among samples to which foreign oil has not been added. It appears, therefore, that this new factor can be of use in determining the presence of added oil, and though it can not be considered as conclusive evidence by itself, when taken in connection with the amount of ether extract and the iodin number of the ether extract it is to be regarded as a factor of considerable value.

REPORT ON FATS AND OILS.

By H. S. Bailey, *Associate referee.*

Collaborative Work on the Detection of Beef Fat in Lard.

The work undertaken this year has been along the lines of the detection of small amounts of added beef fat in lard, by means of the glycerids crystallized from ether solutions. Previous work with the Belfield test,[1] which is now provisional, has indicated that, except in the hands of those who have had considerable experience, it is unsatisfactory for the detection of small amounts of the adulterant.

In 1908 J. E. Emery proposed the substitution of the determination of the melting point of the glycerid crystals in place of their microscopic appearance, as a method for detecting the presence of beef fat.[2] In the hope that this latter method would be found more satisfactory than the Belfield test, the following samples and directions were sent to 15 collaborators:

Sample No. 15, lard + 3 per cent beef tallow.
Sample No. 16, lard + 5 per cent beef tallow.

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[1] U. S. Dept. Agr., Bureau of Chemistry Bul. 107, Rev., p. 147.
[2] U. S. Dept. Agr., Bureau of Animal Industry Cir. 132.

Sample No. 17, lard + 10 per cent beef tallow.
Sample No. 18, lard + 10 per cent lard stearin.
Sample No. 19, pure lard.
Sample No. 20, lard + 5 per cent oleo.

DIRECTIONS.

Technique of the method.—Weigh 5 grams of warm, filtered fat, within 0.1 gram, into a glass-stoppered 25 cc cylinder which should be about 18 mm inside diameter. Add warm ether up to the 25 cc graduation, stopper, shake vigorously until solution is complete, then allow cylinder and contents to stand at a temperature of about 18° C. for 18 hours. A slight variation in temperature makes no difference in the results; but the control, which should be run with pure lard each time a doubtful sample is to be tested, must, of course, be crystallized at the same temperature as the sample in question.

After standing the 18 hours, more or less, decant the supernatant ether from the crystallized glycerids, which will usually be found in a compact mass at the bottom of the vessel. Then add 5 cc of cold ether to the crystals in the cylinder, care being taken not to break up the deposit, and decant. This is repeated three times. The last time the crystals should be loosened from the cylinder and transferred to a small filter paper. Wash the crystals with successive small portions of cold ether until 15 to 20 cc have been used, then by means of suction remove the residual ether quickly and transfer the filter paper with its crystals, after having broken up any large lumps of glycerids by gentle pressure, to a desiccator, where they will dry quickly.

The melting point of these glycerids should be determined in an apparatus similar to that indicated on page 134 of Bulletin 107, Revised. The large test tube is approximately 150 by 25 mm and should be about half filled with water. The bulb of the thermometer, with a melting point tube having an internal diameter of about 1 mm, sealed at one end, containing the crystals, is immersed in the water. The test tube is then placed by a beaker of water, care being taken that the surface of the liquid in both test tube and beaker is about the same level. Heat the water in the beaker rapidly to 55° C. and maintain it there until the thermometer carrying the melting-point tube registers a little above 50°. Raise the heat again until the temperature of the outer bath is about 67° C., then remove the lamp and note the temperature at which the glycerids melt to give a perfectly clear transparent liquid. The amount of crystals used for each determination should be approximately the same each time and ought to occupy a space about 9 mm long and be suspended directly opposite the bulb of the thermometer.

In performing this method great care must be exercised in the preparation of the sample. The presence of water and small particles of extraneous matter or the incomplete solution of the fat in the ether may interfere with the process of crystallization, making it too rapid, so that a large mass of small, fluffy crystals, instead of a compact mass of larger ones, as desired, is obtained. The temperature of crystallization should not be less than 15° C. or more than 20°.

Suggestions to collaborators.—It is suggested that collaborators make mixtures of, say, 3, 5, 10, and 20 per cent of these adulterants with the pure lard and from a determination of the melting point of the mixed glycerids in comparison with those obtained from the pure lard, state what, in their opinion, is the minimum amount of each foreign fat that can be detected by the Emery method; also what is the limit with the Belfield test, and, of course, which of the unknowns are pure lards and which adulterated.

The referee will be glad to receive such suggestions and modifications in regard to these methods as seem desirable; but in order to give them, as they stand, a fair test, it will be necessary to carry through one set of determinations exactly as directed. It will also be of value if other samples of lards of known purity be submitted to these tests by collaborators who are in a position to secure such.

TABULATED RESULTS.

Very few results having been received by August 9 and several of the collaborators having indicated that the amount of time which they would be able to give to the work was limited, a further letter was sent out suggesting that if there were not time for all the work originally outlined, the Emery method only should be tested on the unknown samples. In reply to this the following results were received:

TABLE 1.—*Melting points of glycerids.*

Collaborator.	Sample No.— 15	16	17	18	19	20	Lard.	Tallow.	Lard stearin.	Oleo stearin.
R. H. Kerr	63.6	63.4	62.8	64.0	64.2	62.2	63.6	59.2	63.8	60.0
R. R. Henly	63.4	63.2	62.6	63.9	63.8	62.0	....	....	....	....
R. C. Kent	64.6	64.6	63.8	64.9	65.0	63.4	64.8	59.6	....	59.8
C. P. Wilson	63.0	62.6	62.4	63.0	63.6	61.8	63.6	58.6	62.8	59.2
H. S. Bailey	63.8	63.6	63.0	64.0	64.1	63.1	....	....	....	....
C. W. Harrison	63.7	63.6	63.7	64.0	63.6	62.6	64.2	....	....	....
H. D. Poore	63.8	63.8	63.1	64.0	64.3	63.1	63.8	60.5	63.9	60.7
W. B. Smith	....	63.2	63.0	64.1	64.1	62.5	63.9	59.3	63.7	59.2
L. B. Burnet	64.0	64.1	63.2	64.2	64.2	63.0	64.1	....	....	....
B. B. Schneider	62.8	62.7	61.8	63.2	62.8	61.9	63.6	59.2	63.3	....

TABLE 2.—*Differences between pure lard and sample.*

Collaborator.	Sample No.— 15	16	17	18	19	20	Tallow.	Lard stearin.	Oleo stearin.
B. B. Schneider	−0.8	−0.9	[1] −1.8	−0.4	−0.8	−1.7	−4.4	−0.3	....
R. H. Kerr	.0	− .2	− .8	+ .4	− .6	−1.4	−4.4	+ .2	−3.6
R. R. Henly [2]	− .4	− .6	−1.2	+ .1	....	−1.8	....	....	....
R. C. Kent	− .2	− .2	−1.0	− .1	[1] +1.2	−1.4	−5.2	....	−5.0
C. P. Wilson	− .6	−1.0	−1.2	− .6	.0	−1.8	−5.0	− .8	−4.4
H. S. Bailey [2]	− .3	− .5	−1.1	− .1	....	−1.0	....	....	....
C. W. Harrison	− .5	− .6	− .5	− .2	− .6	−1.6	....	....	....
H. D. Poore	.0	.0	− .7	+ .2	− .5	− .7	−3.3	+ .1	−2.9
W. B. Smith	....	− .7	− .9	+ .2	+ .2	−1.4	−3.6	− .2	−3.7
L. B. Burnet	− .1	.0	− .9	+ .1	+ .1	−1.1	....	....	....
Average	− .32	− .47	− .92	− .04	− .22	−1.39	....	....	....

[1] Omitted from average.

[2] Unknown No. 19, which was same as lard sent out taken for calculating difference.

TABLE 3.—*Results showing judgment of collaborators on purity of samples.*

Analyst.	No. 15. 3 per cent tallow.	No. 16. 5 per cent tallow.	No. 17. 10 per cent tallow.	No. 18. 10 per cent lard stearin.	No. 19. Pure lard.	No. 20. 5 per cent oleo.
R. H. Kerr	Pure	Small adulteration.	4 or 5 per cent.	Pure	Pure	Large amount.
R. R. Henly	do	Adulterated.	More adulteration.	do	do	Adulterated.
R. C. Kent	Doubtful	Doubtful	Adulterated.	do	do	Do.
C. P. Wilson	Adulterated [1]	Adulterated [1]	Adulterated [1]	do [1]	do [1]	Do. [1]
	do	do	do	Adulterated.	do	Do.
L. B. Burnet [2]	Pure	Pure	do	Pure	do	Do.
H. S. Bailey	Doubtful	Adulterated.	do	do	....	Do.
C. W. Harrison	do	do	Doubtful	Pure	Adulterated.	Do.
H. D. Poore [2]	Pure	Pure	Adulterated.	do	Pure	Do.
A. F. Seeker	Adulterated [1]	do [1]	do [1]	Adulterated [1]	Adulterated [1]	Do. [1]
W. B. Smith	....	Adulterated.	do	Pure	Pure	Do.
Do	....	do	do	do	do	Adulterated.
B. B. Schneider	Adulterated.	do	do	do	Adulterated.	Large amount. Adulterated.

[1] Belfield test.

[2] Calculated, by referee, on assumption that a difference of −0.2° indicates doubtful purity and that a difference of −0.5° shows undoubted adulteration.

COMMENTS OF COLLABORATORS.

A. F. Seeker, New York Food Inspection Laboratory: An examination of the samples sent by the referee, following the directions given under the Belfield test in Bulletin 107, Revised, showed that the lard and lard stearin crystallized from ether in similar form, while most of the crystals obtained from the oleo-stearin and tallow (which also resembled each other) differed from the first two and could readily be distinguished from them under the microscope. Even in the case of pure lard, however, there were a few crystal groups which could not be distinguished from some of the tufts found in pure oleo stearin, so that upon the examination of mixtures of lard and oleo stearin and of the samples numbered 15 to 20, it was in many cases found impossible to tell with absolute certainty whether the characteristic beef stearin crystals were present or not. I feel that the Belfield test in its present form is unreliable, and report the results obtained without confidence in their accuracy.

Homer D. Poore, New York Food Inspection Laboratory: It was found that the melting point varied directly with the amount of oleo stearin present up to 10 per cent, but the 10, 20, and 40 per cent mixtures gave the same melting point. The melting points obtained with the 3 and 5 per cent mixtures show that a difference of 1 per cent of oleo stearin changes the melting point from 0.2° to 0.3°. Therefore an 8 per cent mixture would probably have the same melting point as a 10 per cent. The conclusions arrived at from these results are that amounts of oleo stearin in lard up to 8 per cent may be detected within 1 per cent, while amounts over 8 per cent will give the same melting point, which shows adulteration but gives no indication of the amount of adulteration. A 10 per cent mixture of tallow gave a melting point which showed no adulteration, while a 20 per cent mixture gave one that did.

Paul Rudnick, Armour & Co.: We did quite a lot of work on both the Emery and Belfield methods, and in addition to that, some by the so-called Stork method; but the results were absolutely unsatisfactory, chiefly because of a lack of facilities for maintaining the correct temperature.

R. H. Kerr, Bureau of Animal Industry: The slight difference noted between Mr. Henley's results and my own are due to a slightly lower temperature of crystallization which he employed. The temperature for my work was between 17° and 18° C.

B. B. Schneider, Bureau of Chemistry: It would be better, in my opinion, if in the determination of the melting points the temperature of the outer bath were held at a maximum of 65° or 66° C. for high melting-point mixtures. When held at 67° C. the rise of temperature of the inner bath is too rapid for accurate reading.

SUMMARY OF RESULTS.

Summarizing in brief the results tabulated in Table 2:

On sample No. 15, which contained 3 per cent of tallow, of the 9 collaborators reporting, 4 were incorrect, 3 doubtful, and 2 correct, in their judgment of the purity.

Similarly with No. 16 containing 5 per cent of added tallow, 11 reports were received of which 2 were wrong, 1 doubtful, and 8 correct.

On sample No. 17, containing 10 per cent of tallow, 11 reports were received, of which none was wrong, 1 was doubtful, and 10 were correct.

On sample No. 18, containing 10 per cent of lard stearin, which while not lard in the true sense is still a hog fat and, therefore, not detectable by either of the methods tried, 11 reports were received, 1 being incorrect, 10 correct.

Sample No. 19, which was the same pure lard as that sent for comparison, was reported by 2 collaborators as adulterated while 8 were correct in their judgment.

Sample No. 20, containing 5 per cent of oleo, was in every case reported as adulterated.

The tabulated results and the comments of various collaborators indicate a wide difference of opinion as to the value of the proposed method. That it can be carried out with much more facility and less expensive apparatus, no microscope being needed, than the Belfield test, there is no doubt, and as it has been in constant use in the Bureau of Animal Industry for the past two or three years and has given full satisfaction, I think it wise that steps be taken to have it adopted as provisional. Further work, however, should be done on this method next year, and it is hoped that with this year's experience attention can be confined to a narrower field of investigation, so that the time which it will be necessary for collaborators to give to future work will be very small.

REPORT ON THE ADULTERATION OF DAIRY PRODUCTS.

By A. E. PAUL, *Associate Referee.*

At last year's meeting, further study of the Roese-Gottlieb method, as applied to ice cream, milk powders, malted milk, milk chocolate, and cheese was recommended. This work, however, naturally falls to the referee on dairy products, and there was no recommendation properly coming under the head of adulteration.

Looking over this field, the referee was struck by the possibilities for fraud introduced by the " homogenizer," which is just coming into use. This machine enables the unscrupulous manufacturer not only to substitute process butter and skimmed milk for cream, but to " fill " any or all dairy products with whatever foreign fat he chooses to employ. The difficulty of incorporating such adulterants has, up to the present time, practically prevented such practice.

The study of the examination of fats, unmixed with other ingredients, has been very thoroughly carried on by the referees on that subject. However, the separation of sufficient fat for a suitable examination, from such products as ice cream, condensed milk, evaporated milk, cream and fresh milk, presents certain difficulties. At least 7 to 10 grams are required, and from a given sample, the fat should be separated quantitatively or nearly so, in order to eliminate the possibility of a selective separation of fatty bodies. Any chemical alteration of the fat must be avoided and oxidation must be guarded against.

In working out a suitable method several attempts proved unsatisfactory. One of these, a wet method based upon the Roese-Gottlieb method, was fairly successful, but had to be discarded because of the large amounts of inflammable materials involved, which would render the operations exceedingly dangerous. The method to be described appears to be in every way satisfactory. The separation of the fat is practically quantitative, the time consumed in carrying out the operation is insignificant, and the amount of volatile solvent used is very small indeed.

Unfortunately, circumstances made it impossible to carry out this work until rather late in the year. The method was submitted to the collaborators with the request that it be tried on samples procured in the open market and a report returned, this to include the determination of fat by the Roese-Gottlieb method, and the percentage of fat recovered by the method submitted.

Owing no doubt to the lateness of the request, no returns were received. My own results on three products examined were as follows:

Vanilla ice cream:		
Fat, Roese-Gottlieb	per cent	7.58
Fat recovered from 100 grams of sample	grams	7.61
Evaporated milk:		
Fat, Roese-Gottlieb	per cent	7.83
Fat, recovered from 100 grams of sample	grams	7.68
Condensed milk (sweetened):		
Fat, Roese-Gottlieb	per cent	9.03
Fat, recovered from 100 grams of sample	grams	8.95

It is recommended that the method, which reads as follows, be further studied:

Method of extracting fat from cream, ice cream, evaporated milk, and sweetened condensed milk.—Into a 1,000-cc beaker weigh 100 grams (cream, 50 grams) of the material. Add 300 cc water, mix thoroughly, and bring to a boil. Then add, while boiling, very gradually, 25 cc of Soxhlet's copper sulphate solution, diluted with 100 cc water.

In a Büchner funnel wet a filter of suitable size and of loose texture. Filter with suction and wash three times with a little boiling water. Filter as dry as possible. Remove the cake, which should be dry enough to be broken up easily between the fingers. Break into small particles and dry in the open air overnight. Grind in a mortar with a sufficient amount of anhydrous copper sulphate (usually 25 grams is enough) and let stand for a few minutes, or until the product seems quite dry and not at all lumpy.

Put a layer of anhydrous copper sulphate in the inner tube of a large extractor and then add the powdered mixture. Place a loose plug of cotton on top of the mixture and extract with ordinary ether. The ether should be poured into the extractor and allowed to percolate through before the heating is begun. Approximately 50 cc of the solvent will be required. Dry and weigh the fat.

REPORT ON CEREAL PRODUCTS.

By H. L. WHITE, *Associate Referee.*

INTRODUCTION.

At the meeting of this association held in 1910 it was recommended, "That the associate referee on cereal products be instructed to devote special attention to methods for analyzing and testing wheat and flour."

Plan of work.—As no methods for the analysis of cereal products are included under that head in Bulletin 107, Revised, it was deemed advisable by the associate referee to try out, as far as possible, all methods in general use by members of this association and others interested in this line of investigations. Accordingly, after correspondence with several members of the association, methods were collected from various sources and sent, together with two samples of flour, to each of the 10 chemists who offered to collaborate in this work.

Samples.—Sample A was a straight flour from Fife wheat of the 1909 crop, grown in the Red River Valley, near Fargo, N. Dak., and had been in storage in the elevator of the mill at the North Dakota agricultural experiment station until milled March 29, 1911. Sample B was a patent flour from durum wheat, Arnautka variety, of the crop of 1909, grown at Edgeley, N. Dak. The wheat was in storage in the same elevator as sample A until milled March 29, 1911.

METHODS.

The statements in parentheses indicate source or by whom suggested.

MOISTURE.

Method A.—(Adapted from Leach's Food Inspection and Analysis.) Weigh out 5 grams of flour into a flat-bottomed dish, and dry five hours in a water oven at temperature of boiling water.

(NOTE.—Flat-bottomed dishes of aluminum are excellent for use in this method.)

Method B.—(Bul. No. 122, pp. 53–54.) Dry a convenient quantity of flour, approximately 5 grams, at the temperature of boiling water, in a current of dry hydrogen or in vacuo, until it ceases to lose weight.

Method C.—(H. P. Bassett, Bul. No. 122, pp. 55–58.) After treatment with ether (see ether extract, Bassett's method), dry the residue for five hours in a water oven. The loss in weight represents both ether extract and moisture. From total loss in weight per cent subtract the per cent of ether extract.

ASH.

Method A.—(A. L. Winton.) Burn 5 grams of flour in an electric muffle furnace below redness. In case an ash free from carbon is not obtained, add a few drops of nitric acid, evaporate on a water bath, and place again in the muffle furnace for a few moments, repeating the operation two or three times if necessary.

Method B.—(Bul. No. 122, p. 54.) Char a convenient weight of the original sample, approximately 5 grams, in a platinum dish, in a muffle, at the lowest possible temperature until free from carbon. If carbon-free ash can not be obtained owing to its fusibility, cool, add a small quantity of water, warm slightly, drain the more soluble portions of the ash from the carbonaceous residue, carefully dry, and reincinerate to constant weight.

NOTE.—A few drops nitric acid may be added, but if so the ash must be carefully fused until all evolution of gases ceases or slightly high results are likely to be obtained.

Method C.—(Adapted from 2 (*b*), p. 21, Bul. 107, Revised.) Weigh out 5 grams of flour into a porcelain crucible, moisten thoroughly with 10 cc of calcium acetate solution and dry on a hot plate. Heat over an ordinary burner, with low flame, until ash is white, then use blast lamp one minute, cool and weigh. Subtract weight of calcium oxid from total weight. The calcium acetate solution is made by dissolving 1.8 grams precipitated calcium carbonate in pure acetic acid and diluting to 1 liter. Standardize by evaporating 10 cc of the solution to dryness and treat as an ash.

ETHER EXTRACT.

Method A.—(Bul. No. 122, p. 54.) Extract a convenient quantity of the product (from 4 to 5 grams) as dried in the determination of moisture with anhydrous, alcohol-free ether, for 24 hours (with fine flour the addition of an equal weight of clean, dry sand is frequently necessary). Dry the extract at the temperature of boiling water until it ceases to lose weight.

NOTE.—Iodin numbers should be obtained upon the ether extract after purification by solution in petrolic ether, but are best made upon the petroleum ether extract.

Crude ether extract may also be purified with acetone for the determination of the iodin number.

Method B.—(H. P. Bassett, Bul. No. 122, p. 55.) Weigh 10 grams of flour into a tared Gooch crucible of about 20 grams capacity, place in an ordinary Gooch funnel and insert, by means of a two-hole rubber stopper, into the top of a low bell jar resting on a glass plate. Fill the crucible six times with anhydrous alcohol-free ether, drawing off each portion with a filter pump. Collect the ether extract in a bulb flask similar to those used with a Soxhlet apparatus. Connect the bulb with an upright condenser and distil off the ether, using a 32 candlepower lamp as a source of heat. Dry the flask one hour in the water oven, cool and weigh.

ACIDITY OF WATER EXTRACTS OF FLOUR.

Method A.—(Bul. No. 122, p. 54.) Weigh 18 grams of flour into a 500 cc Erlenmeyer flask and add 200 cc of distilled water previously freed from carbon dioxid by boiling in tin and neutralized if need be. Place loosely stoppered flask in a water bath kept at 40° C. for 10 minutes, shaking repeatedly. Remove the flask and allow it to stand, with occasional shaking, at room temperature for one hour. Filter upon dry folded filter, rejecting the first 10 cc and receiving the succeeding 100 cc in a graduate flask. Titrate the filtrate with twentieth-normal sodium hydroxid, using carefully neutralized phenolphthalein in alcohol as an indicator. Each cubic centimeter of twentieth-normal sodium hydroxid represents 0.05 per cent of acidity as lactic acid.

NOTE.—Results obtained with flour at temperatures of 15°, 20°, and 25°, respectively, indicate that the acidity in the solution increases with the temperature. The method outlined above seems to give the maximum acidity.

Method B.—(H. L. White.) Proceed as in Method A, except that the flask should be kept in a water oven maintained at 40° C. for two hours, shaking vigorously every half hour. At the end of the two-hour period decant the liquid portion, shake with 10 to 20 grams of kaolin or clean quartz sand before pouring on filter paper. Express as per cent lactic acid.

SOLUBLE CARBOHYDRATES AS DEXTROSE.

Method A.—(Bul. No. 122, p. 54.) Weigh 16 grams of flour into a 500 cc flask. Add 200 cc of water. Shake occasionally during one-half hour. Filter through a dry folded filter. To 50 cc of the filtrate add 5 cc of concentrated hydrochloric acid. Place the flask in water and invert at 70° C. for 10 minutes. Cool, neutralize, and bring to 100 cc. Filter. Determine the reducing sugars with Fehling solution, by the official method, as described in Bulletin 107, Revised, calculating the reducing sugars as dextrose.

The methods of A. H. Bryan, A. Given, and M. N. Straughn for the determination of sugars (Bureau of Chemistry Circular No. 71) were also recommended for trial by the collaborators. These methods are stated as follows:

Statement of Bryan, Given, and Straughn method for sugars in cereals, etc.

(*a*) *Preparation of solution.*—Place 12 grams of the finely ground material in a 300 cc graduated flask, with 1 to 3 grams of precipitated calcium carbonate to neutralize the acidity, add 150 cc of 50 per cent alcohol by volume (carefully neutralized), mix thoroughly, and boil on steam bath for one hour, using a small funnel in the neck of the flask to condense the vapor. Cool, make to volume (300 cc) with 95 per cent alcohol (neutral in reaction), mix thoroughly, allow to settle. Transfer 200 cc to a beaker with a pipette and evaporate on a steam bath to a volume of from 20 to 30 cc, *but not to dryness.* This should remove all but traces of alcohol. Add 20 cc of water, stir, and transfer the solution to a 100 cc graduated flask and wash the beaker with water into the flask. Add to this enough saturated solution of neutral lead acetate from a burette or pipette to produce complete precipitation, but avoid an excess. Allow to stand 15 minutes and make up to volume of 100 cc with water, shake well, and filter. At least 75 cc of filtrate should be obtained. Add anhydrous sodium carbonate or potassium or sodium oxalate to precipitate all the lead, allow to stand 15 minutes, and pour onto an ashless filter. Test the filtrate for lead with small quantities of the precipitating agents mentioned above and refilter if necessary. This solution represents the sugars from 8 grams of the original material and is used in the following determination:

(*b*) *Reducing sugars.*—Twenty-five cubic centimeters of the filtrate, together with 25 cc of water, are used as the sugar solution for Munson and Walker's method (see p. 241, Bul. 107, Rev.), or 25 cc of the solution can be used for Allihn's method (see p. 49, ibid.). With Allihn's method the amount of dextrose found is multiplied by the factor 1.044 to obtain the equivalent in invert sugar.

(*c*) *Sucrose.*—In a covered 400 cc beaker place 50 cc of the filtrate from (*a*). In case sodium carbonate was used to throw out the lead, add a small piece of litmus paper or neutralize with acetic acid, then add 5 cc of concentrated hydrochloric acid and allow to stand overnight for inversion. Where potassium or sodium oxalate was used for removing lead it is not necessary to neutralize, but the acid can be used direct. At the expiration of the 24 hours neutralize with anhydrous sodium carbonate and wash into a 100 cc flask and make up to the mark. Filter if necessary, and use 50 cc for the determination of total sugars as invert by the method of Munson and Walker or 25 cc by the method of Allihn. The percentage of reducing sugars before inversion calculated as invert sugar is subtracted from the percentage of total invert sugar after inversion, and this product multiplied by 0.95 gives the percentage of sucrose.

For more exact results it is necessary to determine the volume occupied by the 12 grams of material used in this work and to account for it. A large number of determinations have shown the average volume of 12 grams to be 9 cc. Therefore the correction for this would be 0.97, and hence the percentage of original sugars and sucrose should be multiplied by this factor to obtain the true amounts.

GASOLINE COLOR VALUE.

Method A.—(A. L. Winton, see Bul. 137, p. 146, for full statement of method.)

NOTE.—A. S. Mitchell recommends the use of a Kennicult-Sargent colorimeter, using a 0.005 per cent water solution of potassium chromate as a standard.

TOTAL NITROGEN.

Method A.—(Bul. No. 122, p. 54.) Determine the total nitrogen in 2 grams of flour according to the official method, preferably the Gunning method. Bulletin 107, Revised, page 7. The nitrogen times 5.75 gives total proteids.

ALCOHOL SOLUBLE PROTEIDS.

Method A.—(Bul. No. 122, p. 55.) Weigh 4 grams of flour into a 500 cc Erlenmeyer flask, add 200 cc of alcohol, 0.90 specific gravity. Shake occasionally during three hours. Let stand twelve hours. Filter through a dried filter. Evaporate the alcohol from 100 cc of the filtrate after the addition of 5 cc of sulphuric acid and determine the nitrogen as alcohol soluble nitrogen. (This figure less the amid nitrogen gives the alcohol soluble proteid nitrogen or gliadin.)

GLOBULIN AND ALBUMEN (EDESTIN AND LEUCOSIN) AND AMID NITROGEN.

Method A.—(Bul. 122, p. 54.) Weigh 5 grams of flour into a 500 cc Erlenmeyer flask. Add 250 cc of sodium chlorid solution 1 per cent. Stopper and shake thoroughly. Let stand, with occasional shaking, for three hours. Filter on dry paper. Evaporate 100 cc of the filtrate to small volume in a Kjeldahl digestion flask with 5 cc of sulphuric acid. Add remainder of the sulphuric acid and determine the nitrogen by the Gunning method. To a second 100 cc of the filtrate add 5 cc of phosphotungstic acid, 20 per cent solution; shake thoroughly, allow to settle, and filter by decantation. Wash slightly with water. Concentrate the filtrate with 5 cc of sulphuric acid in Kjeldahl flask and determine the nitrogen as amid.

Deduct the amid nitrogen from the nitrogen found in the first fraction to obtain the nitrogen as globulin and albumen. This figure times 6.25 gives globulin and albumen.

GLUTENIN (BY DIFFERENCE).

Method A.—(Bul. No. 122, p. 55.) Deduct from the total nitrogen the salt soluble nitrogen plus the gliadin. This times 6.25 gives the glutenin.

GLIADIN BY POLARIZATION.

Method A.—(Snyder, Bul. No. 122, p. 55.) Weigh 15.97 grams of flour into a 300 cc flask. Add 100 cc of 0.90 specific gravity alcohol. Shake at intervals during three hours and let stand overnight. Filter through a dry folded filter. Polarize in a 220 mm tube. Precipitate the proteids in 50 cc of the filtrate with 5 cc of Millon's reagent. Shake, filter, and polarize the filtrate in a 220 mm tube. Add 50 per cent to the reading and deduct the sum from the first reading. This difference times 0.2 gives the per cent of nitrogen as gliadin.

Method B.—(D. W. Shaw.) Weigh 15.97 grams of flour into a 300 cc flask, add 100 cc of 0.90 specific gravity alcohol. Shake at intervals during three hours and let stand overnight. Filter through a dry folded filter. Polarize in a 200 mm tube. Precipitate the proteids in 50 cc of the filtrate with 5 cc of Millon's reagent. Shake, filter, and polarize the filtrate in a 200 mm tube. Add 10 per cent to the reading and deduct the sum from the first reading. This difference times 0.2 gives the per cent of nitrogen as gliadin.

(NOTE BY A. S. MITCHELL.—Fleurent has devised a modification for gliadin in which an addition of 0.2 per cent of sodium hydroxid is added to the 70 per cent alcohol. In our trials thus far the mixture has been hard to filter, and the modification has not proved very satisfactory. Please also note that various workers are using slightly different percentages of alcohol; for example, Shaw has used specific gravity 0.90. Many workers use 70 per cent by volume. It seems immaterial to me which is used, but I think one or the other ought to be definitely fixed upon.)

DETERMINATION OF MOIST GLUTEN.

Method A.—(Bul. No. 122, p. 54.) Dough up 30 grams of flour with 18 cc of water conveniently in an 8-ounce mortar. Weigh off 16 grams of dough equivalent to 10 grams of flour. Place in water at room temperature for one hour and carefully wash out the starch over boiling cloth or a fine horsehair sieve. After expressing all globules of water, weigh the moist gluten upon a watch glass. Dry in a desiccator for 24 hours and complete drying in water oven.

CRUDE FIBER.

Method A.—(Bul. No. 122, p. 54.) Determine the crude fiber in 2 grams of flour by the official method (Bul. 107, Rev., p. 56), filtering through linen in a Büchner funnel.

Baking test method (*C. H. Bailey*).

Formula: Flour, 340 grams; sugar, 15 grams; yeast, 10 grams; salt, 5 grams, and sufficient water.

Method: All ingredients should first be warmed to a temperature of 32° C., and the fermentation conducted in a regulated fermentation cabinet at the same temperature. Weigh 340 grams of flour into a tight can or box, and place in the fermentation cabinet for at least one hour before mixing. The salt may be weighed into one-half gallon stoneware jars, which are used as receptacles for the dough during the fermentation period, and the sugar weighed into packages which are then ready for use at the proper time. The yeast mixture is prepared as follows: Weigh enough compressed yeast for a baking, plus enough for an extra loaf, into a salt-mouth bottle, and add a quantity of warm, distilled water, equivalent to 30 cc for every 10 grams of yeast taken, and mix the whole thoroughly. This mixture should be prepared at least half an hour before the work of sponging is commenced, being allowed to stand in the fermentation cabinet during the interval; 37.6 cc of this yeast suspension is equivalent to 10 grams of yeast and 30 cc of water, and this quantity is taken in mixing each sponge.

The method of incorporating the materials into the dough is as follows: 140 cc of distilled water (or less if the flour is soft and fluffy in character) and 37.6 cc of the yeast suspension are placed in a stoneware jar, which already contains 5 grams of salt, and into this mixture about two-thirds of the flour, or enough to form a soft sponge, is stirred. The jar is covered, placed in the fermentation cabinet, and allowed to remain there for about 90 minutes.

The sponge is then cut out of the jar and placed in a mixing machine (or on a smooth mixing board, provided no machine is available) together with 15 grams of sugar and the remainder of the flour, which has been kept warm in the meantime. When the Werner-Pfleiderer machine is used, about 200 revolutions of the slow blade are required to thoroughly mix the dough. If the mixing is done on a board, it will require from 12 to 15 minutes hand-mixing to produce a dough of uniform consistency. Water is added a little at a time during the mixing until the dough is of the proper consistency. Judging of the consistency is a matter that requires experience and practice, there being no known device that will determine when the proper condition is reached better than the skilled human hand. When thoroughly mixed the dough is returned to the same jar and again placed in the fermentation cabinet for about 45 minutes, at which time it is removed from the jar and the mass of dough lightly molded and replaced the other side up. In about 20 minutes the dough is again removed from the jar, molded into a loaf, and placed in a well-greased, oblong, black, iron baking pan. This pan is 5½ inches deep, 3 by 5½ inches at the bottom, and 3½ by 6½ inches at the top. The mass of dough is pricked about 10 times with a long needle, placed in the fermentation cabinet, and allowed to rise to nearly the maximum. The time of final rising in the pan varies considerably, but it has been found that hard spring wheat patents and straights usually require from 80 to 100 minutes. All the fermentation periods stated must be varied to suit the flour worked with, and also, to a certain extent, the character and condition of the yeast. Elastic doughs made from flours high in gluten of good quality require a longer fermentation period than do doughs made from flours low in gluten. The figures stated are about the average of the periods allowed hard spring wheat straight flours.

When the dough has reached about the maximum volume it will attain, the pan is placed in the oven, which has been previously heated to 175° C. The temperature should be allowed to rise gradually until at the end of about 5 to 8 minutes it has reached a temperature of from 195° to 200° C. Or, two ovens may be used, one being maintained at about 175° C. and the other at about

200° C., and the loaf transferred to the hotter oven at the end of about 5 minutes, at which time it will have attained its maximum volume. The purpose of employing a lower temperature at the outset is to allow the mass of dough to rise or expand all it will in the oven before the temperature becomes high enough to sear or crust over the outside of the loaf, thus preventing further expansion. The total time of baking varies from about 28 to 45 minutes, depending upon the size of the loaf. Small loaves require more time for thorough baking than do loaves of larger volume.

Since certain factors other than temperature, particularly the quality of the yeast used, influence the volume and quality of bread produced from a given flour, a check loaf should be baked each day from the same check lot of flour, and when there is any marked variation from the average results previously secured in working with this check flour, all tests made at the time should be rejected. When the variation from the average is slight, it is deemed permissible to increase or decrease the recorded volume of other loaves in the same baking, in proportion to the variation of the check loaf from the average.

Recording results: In judging of the baking qualities of the flour, the following factors are of principal importance:

(1) The elasticity or expansibility of the dough, as evidenced by the volume of the loaf.

(2) The color of the flour and of the crumb of the bread produced therefrom.

(3) The "absorption" or quantity of water required to produce a dough of proper consistency.

(4) Other factors, such as flavor, odor, etc.

The absorption is determined during the operation of mixing by noting the quantity of water in cubic centimeters required to produce a dough of the proper consistency. The water used is ordinarily stated in the number of cubic centimeters per 100 grams of flour. This, of course, includes water in the yeast mixture which is added and which is considered as being equivalent to 30 cc.

The volume of the loaf is ascertained by displacement of a suitable material, flaxseed being most convenient for this purpose. The cooled loaf is placed in a box of known volume and the remaining space filled with flaxseed, which is then drawn off into a suitable device and measured. The volume may be stated in cubic centimeters of bread produced from 340 grams of flour or cubic centimenters per 100 grams of flour. The American Society of Milling and Baking Technology also recommend that the volume of bread from 100 grams of dry matter also be recorded.

Color is best judged in the crumb of the finished loaf. In this laboratory the color is expressed on an empirical scale ranging from 50 to 100 or above. The whiter the color the higher the color score, dark flours scoring lower, depending upon the depth of color. A good high-grade patent should score 97 or above on this scale; straight flours 94 or above. The lower or clear flour grades score from 90 on down, depending on their quality and character. It is extremely difficult to convey an idea of the way in which these colors are graded in this laboratory without the use of flour samples whose color score has been determined.

The weight of the finished loaf is also recorded, although this factor is not of so much importance as the quantity of water used per unit of flour. This is true because the weight of the finished loaf is influenced not only by the amount of water used but by the size or volume of the loaf as well. Two doughs to which the same amount of water has been added, but which are raised to different volumes, yield loaves the larger of which will be the lighter. It has been found in working with a large number of samples that the quantity of water used furnishes a better indication of the relative weight of bread which may be produced from a given unit of flour than does the weight of the finished loaf.

Additional remarks concerning abnormalities, such as peculiar odors, flavors, or the like, are included in the report when it is considered necessary. Remarks concerning the crumb texture may also be made, although it is questionable whether this means very much, since the crumb quality may be more nearly a measure of the skill of the operater than of the bread-making qualities of the flour from which it is made. When the dough is sufficiently elastic to produce a loaf of large volume, bread of good quality as regards crumb texture may be produced therefrom in a commercial way.

RESULTS OF ANALYSES.

MOISTURE.

Comparative results obtained by different methods for determining moisture in flour.

Analyst.	Sample A (Fife).			Sample B (durum).		
	Method A (water oven).	Method B (vacuum).	Method C (Bassett's).	Method A (water oven).	Method B (vacuum).	Method C (Bassett's).
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Leila Dunton, Manhattan, Kans	11.01	11.90	11.55	10.52	11.365	11.145
B. R. Jacobs, Washington, D. C		[1] 11.72			[1] 11.12	
L. M. Thomas, Agricultural College, N. Dak	10.13		10.07	9.54		9.65
R. F. Beard, Agricultural College, N. Dak	10.32 [3] 10.91	[2] 11.88	10.09	9.94 [3] 10.91	11.64 [2] 11.47	9.88
C. K. Glycart, St. Paul, Minn	10.24	11.89	9.97	9.55	11.06	9.35
H. L. White, Agricultural College, N. Dak	10.50 10.18	10.56	10.595	10.43 9.65	[2] 10.42	10.53
G. A. Olson, Pullman, Wash	[4] 7.49			[4] 6.34		
Averages	10.47	11.59	10.455	10.076	11.18	10.109

[1] "Drying in a vacuum 4½ hours."
[2] In vacuum oven at 70° C. for periods ranging from 24 to 144 hours.
[3] In aluminum dishes fitted with covers.
[4] Kept in water oven at boiling temperature for 3 hours; not included in average.

Miss Leila Dunton says: "Moisture Method A we do not use in this laboratory for flour samples as it fails to remove all moisture. Method B is very good. The vacuum desiccator we find very convenient and accurate."

These results strengthen the conviction that for accurate results, when working with materials that are affected by the temperature of boiling water, the method utilizing the vacuum oven or desiccator is the most accurate; but this method should be standardized as regards temperature and other conditions. As indicated in the footnotes to the preceding table the time varied from 4½ hours to 144 hours, and the temperature from room temperature to 70° C.

Method C (Bassett's) may well be considered further as a quick method for the approximate determination of both moisture and fat.

ASH.

Comparative results obtained by different methods for the determination of ash in flour.

Analyst.	Sample A (Fife).			Sample B (durum).		
	Method A (electric muffle).	Method B (muffle furnace).	Method C (addition calcium acetate).	Method A (electric muffle).	Method B (muffle furnace).	Method C (addition calcium acetate).
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Leila Dunton, Manhattan, Kans		0.708	0.706		0.69	0.688
L. M. Thomas, Agricultural College, N. Dak			.647			.702
H. L. Wessling, Chicago, Ill	0.72			0.69 .70		
B. R. Jacobs, Washington, D. C	.70			.69		
R. F. Beard, Agricultural College, N. Dak			.695			.70
H. L. White, Agricultural College, N. Dak	.71			.718		
C. K. Glycart, St. Paul, Minn	.678	.673	.663	.686	.673	.661
Averages	.702	.69	.678	.697	.681	.688

Miss Leila Dunton says: "Ash Method B gives very satisfactory results, but requires much more careful handling than the calcium acetate method."

A. S. Mitchell writes: "Referring to the ash determination by Method C, if the results in this laboratory are confirmed by those of other experimenters, it would seem to indicate that the addition of a lime salt, instead of increasing the ash content by the fixation of organic phosphorus and sulphur, has resulted in the loss of alkalis by the use of a temperature far above the point of volatilization of potash.

"The results indicate that the use of a muffle furnace is necessary for the most accurate results. Since Methods A and B give practically the same results, there seems to be no necessity for distinguishing between a method in which an ordinary muffle is used and one where an electric muffle is employed.

"In handling a large number of samples, the calcium acetate method (Method C) is very convenient and may well be retained as a provisional method."

ACIDITY OF WATER EXTRACT.

Comparative results obtained by different methods for the determination of the acidity of water extracts of flour.

Analyst.	Sample A.			Sample B.		
	Method A (lactic acid).	Method B (lactic acid).	Other methods (lactic acid).	Method A (lactic acid).	Method B (lactic acid).	Other methods (lactic acid).
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Leila Dunton, Manhattan, Kans	0.29	0.31		0.26	0.29	
B. R. Jacobs, Washington, D. C	.275			.265		
H. L. Wessling, Chicago, Ill.			[1]0.212			[1]0.185
A. L. Winton, Chicago, Ill.			[1].212			[1].180
R. F. Beard, Agricultural College, N. Dak.	.258	.268		.210	.225	
L. M. Thomas, Agricultural College, N. Dak.	.198	.204		.218	.222	
C. K. Glycart, St. Paul, Minn.	.246	.287		.196	.254	
Averages	.253	.267	.212	.230	.248	.183

[1] Method found in Leach's Food Inspection and Analysis, 2d ed., p. 320.

The following comments were made by analysts:

A. L. Winton: I would like to see a careful study made of the effects of temperature, different times of shaking, relative volume of flour and water on the percentage of acidity. Our method has always been to shake 20 grams of flour for 5 minutes with 200 cc of water, allow to stand for a half-hour, filter, and titrate. This work has been done at room temperature and the solution obtained has been for determination of nitrous nitrogen as well as for acidity. As a matter of convenience, I would like to see a method finally adopted which would combine both determinations, that is, that the aliquots from the same solution could be used for determining both nitrous nitrogen and acidity.

Miss Dunton: Acidity Method A seems to me impractical on account of the varying room temperature, and the temperature playing so important a part in this determination.

A. S. Mitchell: Method B for acidity gives slightly higher results and the results are, if anything, more concordant providing kaolin is not used. The kaolin which we used apparently contributed somewhat to neutralizing the acid. Possibly if it had been previously treated with acid, washed, and dried, its addition would prove desirable, but without this treatment it might become a source of error. It does not seem sufficient to shake the kaolin with water and titrate with acid for a blank. Possibly shaking it with a dilute acid and titrating back might determine the proper correction.

There is a slight increase in acidity by Method B in every case where the collaborator has reported results by both Methods A and B; and it would seem that this method should be given further trial particularly with reference to the action of kaolin, or even as to the necessity of its use, as is suggested in the comment of A. S. Mitchell.

ETHER EXTRACT.

Comparative results obtained by different methods for determination of the ether extracts.

Analyst.	Sample A (Fife).		Sample B (durum).	
	Method A (extract 16 hours).	Method B (Bassett's).	Method A (extract 16 hours).	Method B (Bassett's).
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
Leila Dunton, Manhattan, Kans	1.365	1.30	1.31	1.30
B. R. Jacobs, Washington, D. C	1.22	....	1.35	....
L. M. Thomas, Agricultural College, N. Dak	....	1.30	....	1.25
R. F. Beard, Agricultural College, N. Dak	....	1.38	....	1.27
H. L. White, Agricultural College, N. Dak	[1] 1.67	1.245	....	1.21
C. K. Glycart, St. Paul, Minn	[2] 1.40	1.27	1.345	1.205
Averages	1.414	1.299	1.335	1.247

[1] Flour previously dried in vacuum oven.
[2] On hydrogen-dried flour.

These results indicate the general superiority of Method A, but the wide variations suggest that some details of the method may be improved. This method is already official for crude fat in foods and feeding stuffs (Bul. 107, Rev., p. 39), but in its application to flour some detail, possibly the manner of determining the moisture in the flour, seriously affects the result. For reasons already stated under "Moisture," Method B (Bassett's) may well be further considered.

SOLUBLE CARBOHYDRATES AS DEXTROSE.

Comparative results obtained by different methods for the determination of soluble carbohydrates in flour.

Analyst.	Sample A (Fife).			Sample B (durum).		
	Method A (Bul. 122).	Extraction with sodium carbonate.	Extraction with alcohol.	Method A (Bul. 122).	Extraction with sodium carbonate.	Extraction with alcohol.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
B. R. Jacobs, Washington, D. C	2.38	....	1.40	2.95	....	1.78
L. M. Thomas, Agricultural College, N. Dak	2.43	....	....	2.89	....	....
R. F. Beard, Agricultural College, N. Dak	2.365	[1] 2.15	[1] 1.46	2.595	[1] 2.57	[1] 1.918
W. L. Stockham, Agricultural College, N. Dak	2.31	[1] 1.90	[1] 1.39	2.83	[1] 2.21	[1] 1.80
Averages	2.37	2.03	1.42	2.816	2.39	1.83

[1] Method of Bryan et al., Cir. 71.

These results with alcohol extraction are in quite close agreement, and should be given further trial.

GASOLINE-COLOR VALUE.

Comparative results on Winton's method (a).

Analyst.	Sample A (Fife).	Sample B (durum).
	Per cent.	*Per cent.*
Leila Dunton, Manhattan, Kans	1.225	1.57
H. L. Wessling, Chicago, Ill	1.12	1.40
A. L. Winton, Chicago, Ill	1.12	1.42
R. F. Beard, Agricultural College, N. Dak	1.30	1.49
L. M. Thomas, Agricultural College, N. Dak	1.24	1.38
Averages	1.20	1.45

NITROGEN.

Comparative results on total nitrogen, globulin, and albumen, and amid nitrogen.

Analyst.	Sample A (Fife).				Sample B (durum).			
	Total nitrogen.	Protein (N×5.75).	Globulin and albumen nitrogen.	Amino nitrogen.	Total nitrogen.	Protein (N×5.75).	Globulin and albumen nitrogen.	Amino nitrogen.
	Per ct.	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*
Leila Dunton, Manhattan, Kans	2.505	14.4	[1] 0.502	0.082	2.715	15.6	[2] 0.507	0.105
W. L. Stockham, Agricultural College, N. Dak	2.38				2.56			
B. R. Jacobs, Washington, D. C	2.34				2.58			
R. F. Beard, Agricultural College, N. Dak	2.34				2.48			
G. A. Olson, Pullman, Wash	2.472	14.21	.508	.073	2.73	15.70	.494	.116

[1] Reported as 2.886 per cent protein.

[2] Reported as 2.916 per cent protein.

The close agreement between three out of five of these determinations of total nitrogen seems sufficient evidence to warrant making the method official.

Results on alcohol-soluble protein obtained by determining nitrogen in solution (Method A).

Analyst.	Sample A (Fife).	Sample B (durum).
	Per cent N.	*Per cent N.*
Leila Dunton, Manhattan, Kans	[1] 1.406	[2] 1.65
W. L. Stockham, Agricultural College, N. Dak	1.31	1.52
B. R. Jacobs, Washington, D. C	1.33	1.53
G. A. Olson, Pullman, Wash	1.445	1.64
Average	1.37	1.58

[1] Reported as 8.085 per cent protein.

[2] Reported as 9.53 per cent protein.

MOIST GLUTEN.

Comparative results obtained by two methods.

Analyst.	Sample A (Fife).				Sample B (Durum).			
	Method A (Bul. 122, p. 54).		Wiley method modified.[1]		Method A (Bul. 122, p. 54).		Wiley method modified.[1]	
	Moist.	Dry.	Moist.	Dry.	Moist.	Dry.	Moist.	Dry.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Miss Leila Dunton, Manhattan, Kans.	33.7	13.1	....	....	35.5	13.3	....	....
G. A. Olson, Pullman, Wash.	....	....	37.57	13.815	....	....	40.00	15.564

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[1] The wet and dry gluten determinations were made according to the Wiley method—with this difference: 10 grams of flour plus 6 cc of water were mixed instead of 30 grams of flour and 18 cc of water. The wet gluten wads were then placed in the vacuum oven and dried for three hours under 65 mm vacuum and 85° C. temperature. Note: The reduced pressure and temperature combined cause the glutens to expand rapidly, and the resulting large surface exposed under these conditions permits the water to be driven off rapidly.

BAKING TESTS.

Comparison of results obtained by different bakers.

Baker and sample.	Weight flour.	Water absorbed.	Fermentation periods.				In pan.	Weight loaf.	Volume.	Color.	Texture.
			1	2	3	4					
Sample A (Fife):											
	Grams.	*cc.*	*Min.*	*Min.*	*Min.*	*Min.*		*Grams.*	*cc.*		
L. M. Thomas, Agricultural College, N. Dak.	340	196	81	......	40	25	72	490	2,490	98	98
C. H. Bailey, Agricultural College, N. Dak.	340	177	......	......	......	......	......	483	2,520	101	99
Leila Dunton, Manhattan, Kans.	340	192	......	......	......	......	......	473	1,990	[1] 96	[1] 90
B. R. Jacobs, Washington, D. C.	[2] 300	205	90	[3] 60	47	20	65	485	2,135	90	100
	......	[4] 229	......	......	......	......	......	535	2,215	90	100
Sample B (durum):											
L. M. Thomas, Agricultural College, N. Dak.	340	205	90	......	40	25	86	495	2,160	98	97
C. H. Bailey, Agricultural College, N. Dak.	340	186	......	......	......	......	......	494	2,020	91	99
Leila Dunton, Manhattan, Kans.	340	187	......	......	......	......	......	468	1,780	[1] 96	[1] 85
B. R. Jacobs, Washington, D. C.	[2] 300	202	90	[3] 60	45	20	55	488	1,681	95	100
	......	[4] 227	......	......	......	......	......	515	1,672	95	100

[1] Graded in comparison to a loaf baked at the same time from a straight hard winter-wheat flour, which was graded 100 per cent on both color and texture.
[2] Dry flour.
[3] This fermentation period was not included in the original method.
[4] Second analysis by method used in Bureau of Chemistry, see following section.

METHODS OF MAKING BAKING TESTS USED IN THE LABORATORY OF PLANT PHYSIOLOGICAL CHEMISTRY, BUREAU OF CHEMISTRY.[1]

Ferment.—Mix 80 grams of compressed yeast, 80 grams of sugar, and 40 grams of salt in 1,200 cc of water at 30° C. It is best to dissolve yeast in a small amount of this water and add it to the remaining portion. This liquid is kept in the sponge box at 30° C. for one hour.

Dough.—Place 340 grams of flour, previously warmed in a sponge box to 30° C., in a crock, add 150 cc of the yeast mixture and enough water at 30° to make a dough of the standard consistency. The mixing is done with a knife or a larger spatula and requires but one or two minutes' stirring. Place the crock containing this dough in the sponge box for 20 minutes, at the end of which time remove it, scrape the dough with a spatula from the sides of the crock and fold over in the hands 21 times, place in the crock and in the sponge box for 20 minutes; take out and fold over 14 times, again place in the crock and in the sponge box for 20 minutes; take out and fold in the same manner 9 times. Place in the crock and sponge box for another 20 minutes, take out and fold once or twice, place in the baking pan, smooth surface up, prick with a long pin 10 or 12 times, and place in the sponge box and raise to the maximum, which requires from 50 to 70 minutes, according to the kind of flour to be tested; then place in the oven, the temperature of which is 210° C., and bake for 40 minutes. The baking tests are run in sets of seven. There is enough ferment in the above-mentioned quantity for eight bakes. If it is desired to bake a second set of loaves another ferment is started about 70 minutes after the first so as to allow sufficient time between the ferments for the manipulating of the dough.

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[1] By B. R. Jacobs.

After the bread is baked it is allowed to cool for one hour, weighed, and measured, the measurement being done by displacement in a vessel of known volume. using turnip seed to fill that space in the vessel not occupied by the loaf.

ADDITIONAL BAKING TESTS.

G. A. Olson, of Pullman, Wash., submitted the following method and results obtained therewith:

Method.—The baking tests were made on 100 gram lots of flour, which were worked up with a yeast solution containing the requisite amount of water, sugar, and salt, and placed in 300 cc cubical tins for expansion at 32° C. for straight bakes. A second lot was placed in porcelain dishes, covered with watch glasses and allowed to expand at 32° C. for rekneading. After rekneading these doughs were placed in 300 cc cubical tins and allowed to expand a second time, when finally they were baked at 190° C. for 30 minutes. After a lapse of one hour the loaves were weighed and finally put away in a moist chamber and cut the next morning in order to determine the texture and the color of the crumb. Note: The yeast was developed in potato water containing the requisite amount of sugar, salt, and water. The absorption tests were determined by taking definite amounts of yeast solution and working it up with enough flour to make the proper consistency in each case. The amount of yeast solution required for 100 grams of flour was calculated from the difference in the amount of flour used for the absorption determination from a weighed larger amount of flour.

RESULTS OF BAKING TESTS.

Data.	Sample A.	Sample B.
Straight bake:		
Weight of dough when set in oven to bake (grams)	162.75	170.75
Weight of loaf (grams)	138.65	144.20
Volume of loaf (cc)	350.0	330.0
Approximate specific gravity	0.396	0.437
Time for dough to rise (minutes)	110	110
Number cubic centimeters per pound loaf (calculated)	1,190	1,122
Shape of loaf	Good.	Good.
Texture of crumb	Uniform.	Uniform.
Color of crumb	Yellow cast.	Yellow cast.
Rekneaded bake:		
Weight of dough when set in oven to bake (grams)	159.55	147.75
Weight of loaf (grams)	141.95	142.05
Volume of loaf (cc)	380.0	360.0
Approximate specific gravity	0.373	0.394
Time for dough to rise (minutes)—		
First time	110	110
Second time	70	70
Shape of loaf	Excellent.	Good.
Texture of crumb	Slightly open.	Slightly open.
Color of crumb	Yellow cast.	Yellow cast.

There is so much variation in methods and results in the baking tests that no very definite conclusions can be drawn. It is highly desirable, however, that the amounts of the materials used, except water, be the same in all methods. Attention should also be called to the wide variation in moisture content of yeast, sometimes as much as 30 per cent, which, if not taken into account, may affect results considerably.

RECOMMENDATIONS.

It is recommended—

(1) That the method for the determination of moisture stated in Bulletin 107, Revised, page 38 (1), be made official for cereals, and that a further study be made of the efficiency of the vacuum desiccator as compared with the vacuum oven.

(2) That Method B for the determination of ash (Bul. 122, p. 54) be made an official method; and that Method C for the determination of ash (adapted from 2 (*b*), p. 21, Bul. 107, Rev.) be made a provisional method.

(3) That Method B for the determination of the acidity of the water extract of flour be made a provisional method.

(4) That Method A for the determination of the ether extract (Bul. 107, Rev., p. 39, 5b (1)) be made official.

(5) That the methods of A. H. Bryan, A. Given, and M. N. Straughn (Cir. 71, Bureau of Chemistry), for the determination of soluble carbohydrates, be given a more extended trial.

(6) That Method A for total nitrogen (Bul. 122, p. 54) be made official after making the following change: For "2 grams" read "1 to 2 grams."

(7) That Winton's gasoline color value method be made provisional. (See Bul. 137, p. 146.)

(8) That in further trials of baking methods the amounts of materials used, except water, be the same in all methods; and that the water content of the yeast and the fermenting value of the yeast be given due consideration in all methods.

[The following methods and paper bearing on same were submitted subsequent to the meeting and inserted in the Proceedings in accordance with the motion made by Mr. Winton. (See p. 196.)]

DETERMINATION OF NITROUS NITROGEN.

GRIESS-ILOSVAY METHOD.

(1) *Preparation of reagents.*

(*a*) *Sulphanilic acid solution.*—Dissolve 0.5 gram of sulphanilic acid in 150 cc of 20 per cent acetic acid.

(*b*) *Alpha-naphthylamin hydrochlorid solution.*—Dissolve 0.2 gram of the salt in 150 cc of 20 per cent acetic acid with the aid of heat.

(*c*) *Standard sodium nitrite solution.*—Weigh 0.22 gram of dry C. P. silver nitrite into a 1,000 cc flask, add about 40 cc of hot water and agitate gently until the solution is clear. Add a slight excess of C. P. sodium chlorid, shake until the silver chlorid flocks, make up to 1,000 cc, mix, and allow to settle. Draw off 5 cc of the clear solution and dilute to 1 liter; 1 cc of this solution contains 0.0001 mg of nitrogen as nitrite.

(*d*) *Standardization of sodium nitrite solution.*—The sodium nitrite solution may be standardized as follows: Make a solution of sodium nitrite similar to that provided in (*c*), but making the first dilution of five times the concentration (1 cc equals 0.1 mg of nitrogen as nitrite), avoiding material excess of sodium chlorid as a precipitant. Place a measured amount of 50 cc of one-hundredth normal potassium permanganate in a 5-inch porcelain evaporating dish, together with 200 cc of pure water and 25 cc of dilute sulphuric acid (1:4); warm to

50° C. and titrate with the standard solution of sodium nitrite, adding slowly, as the reduction of the permanganate is not instantaneous. One cubic centimeter of hundredth-normal permanganate is equivalent to 0.00077 gram of silver nitrite, hence 50 cc of the permanganate should be decolorized by exactly 35 cc of standard nitrite solution. (See Krauch, Chemical Reagents, Their Purity and Tests, p. 188.)

(2) *Determination.*

(*a*) *Method I.*—Select a series of 100 cc volumetric flasks of uniform dimensions and color. Place 2 grams of high-grade nitrite-free flour in each; add approximately 70 cc of nitrite-free distilled water and shake until the flour is thoroughly moistened.

From a burette containing the standard nitrite solution (*c*), add to the flasks containing the flour and water amounts of solution increasing by definite increments to form a series of standards for comparison having a range covering the probable nitrite content of the unknown sample. One flask should be reserved for a blank test. Where the quantity of nitrite present is small, the nitrite solution in the flasks may be increased by 0.4 cc each. Where bleaching is excessive, 1 gram of flour may be used throughout, or the standards may be given a wider variation in nitrite content.

In order to avoid making a large series of standards it is well to make a preliminary test to ascertain the approximate nitrite content of the unknown.

To each of two similar flasks add 2 grams of the flour to be tested and 90 cc of water; shake thoroughly and digest all of the flasks, including the blank, in a water bath at 40° C. for at least 15 minutes; add 2 cc of each of the Griess reagents, "(*a*)" and "(*b*)," to each flask. Continue the digestion at 40° C. for at least 20 minutes. The color should be developed in all flasks under conditions as nearly uniform as possible.

Make up to the marks with nitrite-free water and compare the unknown with the series of standards. This may best be done in a large, white, enameled pan, the effect of the turbidity, due to the flour, being minimized by the white background. The solutions should be allowed to subside and should not be shaken during comparison.

(*b*) *Method II.*—Weigh 20 grams of the flour into a 500 cc Erlenmeyer flask, add 200 cc of water, free from nitrites, previously warmed to 40° C., and close the flask with a rubber stopper. Shake vigorously for five minutes and digest for one hour in a water bath, keeping the temperature of the liquid in the flask at 40° C., and shaking at 10-minute intervals. Finally filter on a dry-folded filter free from nitrites. As the first portion of the filtrate is turbid, it should be returned to the filter and the operation repeated until a clear liquid is secured. Pipette 50 cc of the filtrate and, at the same time, 50 cc of the standard nitrite solution (*c*) into small flasks; add to each 50 cc of water 2 cc of solution (*a*) and 2 cc of solution (*b*), shake, and allow to stand one hour to bring out the color. Compare the two solutions in a colorimeter. Divide the height of the column of the standard solution by that of the solution of the sample, thus obtaining the parts of nitrogen as nitrous acid (free and combined) per million of flour.

A blank test should be made to establish the absence of nitrites in the water and filter paper, and care taken throughout the process to avoid contamination from nitrous fumes, gas flames, etc.

Practically the same results will be obtained if the digestion is made at 20° C. or at room temperature, but the method of solution as described above permits the determination of acidity by Mitchell's method in an aliquot of the same solution, provided the water used has previously been boiled in a Jena flask or narrow-mouthed tin vessel for five minutes or until neutral.

THE EFFECTS OF TIME AND TEMPERATURE OF DIGESTION ON THE AMOUNTS OF ACIDITY AND NITROUS NITROGEN EXTRACTED FROM FLOUR.

By A. L. Winton and A. W. Hansen.

Mitchell has shown that the results on the acidity of flour increase with the temperature of digestion up to 40° C. The following experiments were made to determine the effects of time, as well as temperature, on acidity determinations, and also to learn the influence of these factors on the amounts of nitrites extracted. The purpose of the work was to learn whether both acidity and nitrous nitrogen can be determined in aliquots of the same solution.

METHODS.

METHODS OF SOLUTION.

Method 1.—Digest at 20° C. for one hour, shaking at the start. To 20 grams of flour contained in a 500 cc Erlenmeyer flask, add 200 cc of distilled water at 20° C., free from nitrites and previously boiled in a Jena flask until free from acidity. Stopper tightly, shake for five minutes, and allow to stand for one hour at 20° C. Decant the liquid on to a folded 24 cm S & S No. 588 filter paper, taking care not to disturb the flour at the bottom of the flask. Pass the first portion of the turbid filtrate through the paper a second time and repeat this operation until a clear filtrate is secured.

Method 2.—Digest at 20° C. for one hour, shaking at intervals. Proceed as in method 1, shaking the flask vigorously at 10-minute intervals in addition to the five minutes' shaking at the start.

Method 3.—Digest at 20° C. for two hours, shaking at intervals. Proceed as in method 2, except that the digestion is continued for two hours, shaking at 10-minute intervals.

Method 4.—Digest at 40° C. for one hour, shaking at intervals. Proceed as in method 2, except that water at 40° C. is employed and the digestion is carried on in a water bath at 40° C.

Method 5.—Digest at 40° C. for two hours, shaking at intervals. Proceed as in method 4, except that the digestion is continued for two hours, shaking at 10-minute intervals.

Method 1 is the same as described in Leach, Food Inspection and Analysis, second edition, page 322, except that the definite temperature, 20° C., is employed instead of room temperature. It is also essentially the same as described by Jago, in The Technology of Bread Making, page 768, except that the time of standing is one hour instead of one-half hour.

Method 4 is the same as that suggested by Mitchell, except that the directions as to shaking are more specific, and the digestion is conducted throughout at 40° C. instead of 10 minutes at 40° C. and the remainder of the time at room temperature.

DETERMINATION OF ACIDITY.

Titrate 50 cc of the clear filtrate, prepared as described in the foregoing sections, with tenth-normal phenolphthalein solution, using phenolphthalein as an indicator.

DETERMINATION OF NITROGEN AS NITROUS ACID.

Remove another portion of 50 cc of the clear filtrate and proceed as described on page 114, Method II.

RESULTS OF ANALYSES.

The determinations of acidity and nitrous nitrogen were made by the foregoing methods on four samples of bleached flour, two of hard wheat, heavy and light bleached, and two of soft wheat, heavy and light bleached. The results are given in the accompanying table.

It appears that for the determination of acidity 40° C. is the most satisfactory temperature of extraction, not only because the results are higher, but because this is the only temperature that is practicable in all laboratories and in all seasons. Digestion for one hour gave practically the same percentages of acidity as digestion for two hours, provided the flask was shaken at 10-minute intervals during the digestion

The amount of nitrous nitrogen extracted was the same for digestion at 40° C. as for 20° C., and for one hour as for two hours; furthermore, shaking at the start extracted as much as shaking at intervals.

From the results obtained, method 4 appears to be the most practicable for the determination of acidity, and aliquots of this solution may be used for the determination of nitrous nitrogen.

Effects of time and temperature of digestion on the amounts of acidity and nitrous nitrogen extracted from flour.

Method.	Hard wheat, patent.				Hard wheat, clear.			
	Light bleached.		Heavy bleached.		Light bleached.		Heavy bleached	
	Acidity as lactic acid.	Nitrogen as nitrous acid.	Acidity as lactic acid.	Nitrogen as nitrous acid.	Acidity as lactic acid.	Nitrogen as nitrous acid.	Acidity as lactic acid.	Nitrogen as nitrous acid.
	Per cent.	*Parts per million.*	*Per cent.*	*Parts per million.*	*Per cent.*	*Parts per million.*	*Per cent.*	*Parts per million.*
Digested at 20° C:								
1-hour digestion—								
Shaken at start	0.072	0.40	0.077	3.04	0.165	0.32	0.198	2.92
Shaken at intervals	.113	.42	.113	3.00	.243	.30	.263	3.00
2-hour digestion, shaken at intervals	.108	.42	.113	3.04	.248	.30	.267	2.96
Digested at 40° C:								
1-hour digestion, shaken at invals	.126	.40	.126	3.04	.293	.32	.293	2.92
2-hour digestion, shaken at intervals	.126	.42	.131	3.04	.306	.32	.303	2.92

Method.	Soft wheat, patent.				Soft wheat, clear.			
	Light bleached.		Heavy bleached.		Light bleached.		Heavy bleached.	
	Acidity as lactic acid.	Nitrogen as nitrous acid.	Acidity as lactic acid.	Nitrogen as nitrous acid.	Acidity as lactic acid.	Nitrogen as nitrous acid.	Acidity as lactic acid.	Nitrogen as nitrous acid.
	Per cent.	*Parts per million.*	*Per cent.*	*Parts per million.*	*Per cent.*	*Parts per million.*	*Per cent.*	*Parts per million.*
Digested at 20° C:								
1-hour digestion—								
Shaken at start	0.090	0.40	0.090	2.72	0.162	0.32	0.153	2.28
Shaken at intervals	.117	.38	.117	2.72	.189	.34	.202	2.28
2-hour digestion, shaken at intervals	.122	.38	.122	2.68	.196	.32	.212	2.28
Digested at 40° C:								
1-hour digestion, shaken at intervals	.131	.40	.131	2.72	.207	.34	.225	2.32
2-hour digestion, shaken at intervals	.131	.38	.136	2.72	.216	.34	.230	2.32

A RECALCULATION OF JUCKENACK'S EGG-NOODLE TABLES.

By R. W. Hilts.

The work of Juckenack[1] on the analysis of egg noodles and the estimation of their egg content by the determination of lecithin phosphoric acid has received quite general recognition. His tables for estimating the number of eggs to the pound of flour can not, however, be used without restriction for the English weight system. Although the author does not so state, an examination of the formulas by which the tables were calculated shows that the basis is a so-called "pound" of 500 grams. Though the difference is insignificant in the lower figures of the tables, it would lead to some error with noodles containing much egg. For convenience Juckenack's tables have accordingly been recalculated on the basis of the avoirdupois pound of 453.59 grams, using otherwise the same formulas and fundamental values.

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[1] Zts. Nahr. Genussm., 1900, 3: 1, 11.

Recalculated tables.

For use with whole eggs.					For use with yolks.				
Number of eggs to 1 pound of flour.	Average data on dry matter of such noodles.				Number of yolks to 1 pound of flour.	Average data on dry matter of such noodles.			
	Ash.	Phosphoric acid.		Protein (N×6.25).		Ash.	Phosphoric acid.		Protein (N×6.25).
		Total.	Lecithin.				Total.	Lecithin.	
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*		*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
0	0.460	0.230	0.0225	12.00	0	0.460	0.230	0.0225	12.00
½	.518	.253	.0385	12.55	½	.475	.253	.0388	12.20
1	.575	.275	.0541	13.08	1	.491	.276	.0548	12.41
2	.684	.318	.0840	14.11	2	.521	.321	.0858	12.80
3	.786	.359	.1120	15.07	3	.550	.364	.1157	13.18
4	.883	.397	.1385	15.97	4	.578	.405	.1445	13.54
5	.974	.433	.1636	16.83	5	.604	.445	.1722	13.89
6	1.061	.468	.1874	17.65	6	.630	.483	.1990	14.22
7	1.143	.500	.2099	18.42	7	.655	.520	.2248	14.55
8	1.221	.531	.2314	19.15	8	.680	.556	.2497	14.87
9	1.295	.560	.2517	19.85	9	.703	.591	.2738	15.17
10	1.366	.588	.2711	20.52	10	.725	.624	.2971	15.47

It was necessary to find by calculation from the original tables the basic values assumed for ash and protein in the egg yolk and whole egg. These were found to be based on the averages of König,[1] as are all the other values for the egg with the exception of total and lecithin phosphoric acid, which Juckenack took from his own work. The flour is assumed to contain an average of 13.05 per cent of moisture, with dry matter of the composition shown at the top of the tables.

NOTE ON DETERMINATION OF TIN IN FOODS.

By A. W. Hansen and L. C. Johnson.

The writers do not wish to claim credit for any new principles in the method here outlined; all available information was utilized. The use of nitric and sulphuric acid to carbonize the organic matter is essentially the procedure followed in the Marsh-Berzelius-Sanger arsenic method, and in the Smith-Bartlett[2] tin method, except that dilute acids are used, which greatly reduces the danger of loss from frothing. A description of the method follows:

Method of making solution.—Place in a Kjeldahl flask an amount of the sample containing about 25 grams of solid matter. Add some pieces of broken glass, about 200 cc of water, 100 cc of concentrated nitric acid, and 50 cc of concentrated sulphuric acid. Heat cautiously over a wire gauze. Continue alternately adding nitric acid and heating till all organic matter is destroyed, as evidenced by the absence of charring, and boil down the solution to a small volume. Finally add about 25 grams of potassium sulphate and heat till all nitric acid is driven off, adding more sulphuric acid if necessary. This method of making the solution could doubtless be used to advantage in determining copper, lead, and zinc in foods.

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[1] Chemie der Menschlichen Nahrungs und Genussmittel, 4th ed., vol. 1, pp. 98–99; vol. 2, p. 573.

[2] U. S. Dept. Agr., Bureau of Chemistry Bul. 137, p. 136.

Determination of tin.—Transfer the sulphuric acid solution to a liter Erlenmeyer flask, dilute to about 600 cc, and pass in hydrogen sulphid. Let stand overnight. Filter off sulphids and wash with hot water. Digest filter and precipitate with hot sodium sulphid, filter, and wash with hot water.

Barely acidify filtrate from the sodium sulphid treatment with hydrochloric acid. Filter off the tin sulphid, wash with hot water, ignite, and weigh as tin oxid. (Factor for metallic tin, 0.78808.)

REPORT ON CONDIMENTS OTHER THAN SPICES.

By W. J. McGee, *Associate Referee.*

After some correspondence and discussion it was decided to devote the work done under the head of condiments other than spices to ketchup. An outline for the chemical examination of ketchup had been sent to each of the branch laboratories in circular letter from the Bureau of Chemistry, dated February 18, 1911. These methods were as follows:

Statement of Methods.

Total solids.—Determine as usual, using from 3 to 5 grams and drying at the temperature of boiling water for 4 hours. The sample should be spread out in a thin layer for drying.

Insoluble solids.—Wash 20 grams repeatedly with hot water, centrifuging after each addition of water. Pour the clear supernatant liquid through a tared double filter on a Büchner funnel. A cylinder 1 to 1¼ inches in diameter and 5 to 6 inches long is convenient for washing and centrifuging. This may be prepared by shortening a colorimeter tube. After four or five washings transfer the remaining insoluble matter to the filter and finally dry for two hours at 100° C. The filter paper used in this determination should be dried for two hours at 100° before the original weighing.

Soluble solids.—Calculated by difference.

Ash.—Determine in the usual manner. Avoid heating above dull redness.

Alkalinity of ash.—Transfer the ash to a 100 cc flask and dilute to the mark. This solution, together with a small amount of insoluble matter, is shaken thoroughly and an aliquot portion pipetted off as quickly as possible for the determination of alkalinity of the ash. Determine as under "Alkalinity of Insoluble Ash," Bulletin No. 107, Revised, page 69, and report as cubic centimeters of tenth-normal acid per ash of 1 gram of ketchup. Also report as per cent potassium carbonate in the salt-free ash.

Sodium chlorid.—Determine on an aliquot portion of the ash solution by Mohr's method.

Reducing sugars before inversion.—Weigh 10 grams into a 100 cc flask, clarify with an excess of normal lead acetate, dilute to the mark, and filter. Remove the excess of lead with dry sodium sulphate or sodium carbonate, filter, and determine reducing sugars by the Munson and Walker method, Bulletin 107, Revised, page 242. Calculate the sugars as per cent invert sugar and sucrose. (See table in Bul. 107, Rev., pp. 243–251, column 5.)

Reducing sugars after inversion.—Transfer 50 cc of the filtrate obtained in the previous determination to a 100-cc flask, add 5 cc concentrated hydrochloric acid, let stand overnight, neutralize exactly with sodium hydroxid, cool, make to the mark and determine reducing sugars as before. Calculate as invert sugar.

Sucrose.—Calculate as directed on pages 41 and 42 of Bulletin 107, Revised.

Polarization after inversion.—Determine in the usual manner. Use the normal weight of ketchup and allow to stand overnight at room temperature for inversion. Report as polarization on the normal weight, that is, 26 grams in 100 cc.

Total acids as citric.—Use 5 grams and determine as under "Vinegar," Bulletin 107, Revised, page 103. (1 cc of tenth-normal alkali=0.0063 gram of citric acid.)

Volatile acids as acetic.—Determine volatile acids in the usual manner, using 25 grams of sample. (1 cc of tenth-normal alkali=0.006 gram of acetic acid.) Reserve the neutralized distillate for the determination of butyric and formic acids.

Butyric acid.—Evaporate to dryness the neutralized distillate obtained under "Volatile acids," in a weighed platinum dish on the steam bath. Heat for 2 hours in an oven at 100° C. and weigh. From the alkali used and the weight of the salts calculate the per cent of sodium in the salts. Decompose the salts with about 5 cc of 10 per cent sulphuric acid. The acid residue thus obtained should be smelled to ascertain the presence of butyric acid. The sodium content of sodium acetate is 28 per cent, while that of sodium butyrate is 20.5. Owing to the natural limits of error, a lower sodium content than 28 per cent may occur even in the absence of butyric acid, but if the amount of sodium present in the salt is found by the calculation directed above to be as low as 27 per cent, it is our experience that a considerable amount of butyric acid is present. A much smaller amount can be detected by smell, however, and this method of calculation is only of value in determining the approximate amount of butyric acid where considerable quantities are present.

Formic acid.—Test for formic acid as follows: To the salt residue acidified with sulphuric acid obtained in the preceding determination small quantities of magnesium are added, the action of the magnesium upon the acid being allowed to continue for about an hour. Formic acid present is thus reduced to formaldehyde, which may be detected by the Hehner or Leach method. If the reaction is positive the presence or absence of formaldehyde in the original sample should be demonstrated.

Fixed acids as citric.—Calculate by difference.

Lactic acid.—Make 100 grams up to 500 cc with water, mix thoroughly, centrifuge, and take 400 cc of the clear portion. Evaporate this to about 100 cc, add 10 cc of 20 per cent normal lead acetate solution, filter, and wash the precipitate twice with a small amount of water. To the filtrate add a moderate excess of sulphuric acid, filter, wash the precipitate with a small amount of water, and evaporate the filtrate on the steam bath to a convenient volume for extraction in a continuous liquid extractor. In this laboratory this volume is 100 cc. Extract for 18 to 20 hours in a liquid extractor with washed ether. (Ether sufficiently pure for this purpose may be prepared by shaking out ordinary ether once with a sodium hydroxid solution and then 10 times with small quantities of water.) To the ether extract is added about 20 cc of water, and the ether is evaporated. Care must be taken to remove all traces of ether. The water is added before evaporation because of the possible presence of traces of sulphuric acid which might otherwise char the lactic acid. Approximately 3 grams sodium hydroxid are then added to the water solution and 50 cc of a 1.5 per cent solution of potassium permanganate are added from a pipette. This is heated on a water bath at 100° for one-half hour. At the end of that time, or before, if the color is not a decided blue black or purple, but is green or colorless above the layer of brown precipitate, more standard permanganate in measured portions is added until, after heating one-half hour on a boiling-water bath, the color is a blue black or purple. The oxidation is then complete. The hot solution is strongly acidified with dilute sulphuric acid and about 50 cc of 10 per cent sulphuric acid, and standard oxalic acid is run in from a burette until the solution is decolorized. In this laboratory a 5 per cent solution of oxalic acid is used for that purpose. Any slight excess of oxalic acid is titrated back with the same standard permanganate solution. It should be understood that any standard permanganate and oxalic acid solutions may be used within reasonable limits of strength. In alkaline solution the permanganate oxidizes the lactic acid quantitatively to oxalic acid according to the equation:

$$2C_3H_6O_3+10KMnO_4=2(COOH)_2+4H_2O+2CO_2+5MnO_2+5K_2MnO_4$$

In acid solution the oxalic acid is further oxidized by the permanganate to carbon dioxid and water according to the equation:

$$5(COOH)_2+2KMnO_4+3H_2SO_4=10CO_2+8H_2O+K_2SO_4+2MnSO_4$$

Calculation: The total weight of permanganate used in the oxidation of the lactic acid is determined by subtracting the permanganate equivalent of the oxalic acid used from the total amount used. The weight of permanganate times 0.237 equals the weight of lactic acid.

Citric acid.—Weigh 25 grams into a 250-cc beaker, make up to approximately 200 cc with 95 per cent alcohol, allow to stand with frequent stirring for four hours, filter through a folded filter and wash with 50 cc of 80 per cent alcohol, To the filtrate add 10 cc of 20 per cent barium acetate solution, stir well with a glass rod, allow to stand overnight. In the morning filter on a Gooch crucible, washing with 50 per cent alcohol, dry 2 hours in an oven at 100° C. and weigh. Weight of precipitate times 0.49 equals citric acid.

Nitrogen.—Determine as usual.

Protein.—Calculated. (Nitrogen × 6.25.)

Ammonia by distillation with magnesium oxid.—Make 50 grams up to 300 cc with distilled water, add 10 grams magnesium oxid, place in a 1-liter distilling flask, and distil 100 cc in approximately 30 minutes, running the distillate into standard acid. Titrate the excess of acid with tenth-normal sodium hydroxid, using cochineal or methyl orange as an indicator. (1 cc of tenth-normal hydrochloric acid = 0.0017 gram NH_3.)

Nitrogen partition with basic lead acetate.—Weigh into a 250 cc beaker 25 grams of the sample. Make up to 200 cc with water, add 10 cc 20 per cent basic lead acetate, mix thoroughly, filter through a folded filter, and determine nitrogen by the Kjeldahl method in an aliquot portion of the filtrate. For this purpose it is well to use as large an amount of filtrate as possible. Calculate this nitrogen as per cent total nitrogen in the original ketchup. From the total nitrogen of the ketchup calculate the quantity of nitrogen in the basic lead acetate precipitate.

Pectin determination.—Filter the ketchup through a folded filter and weigh 25 grams of the filtrate into a 250 cc beaker. Add 95 per cent alcohol to a total volume of 200 cc. Mix thoroughly, allow to stand overnight. The so-called pectin bodies will probably be found firmly sticking to the sides and bottom of the beaker. If this is the case, pour off the alcohol, dissolve the pectins in a small amount of warm water, transfer to a large platinum dish, make up to approximately 100 cc with 95 per cent alcohol, and allow to stand 12 hours. Pour off the alcohol, dry the pectins contained in the dish for 2 hours in an oven at 100°, and weigh. Ash at a dull red heat, weigh and subtract the weight of the ash. The loss on ignition represents the so-called pectin bodies for a 25-gram sample.

Sand.—Weigh 100 grams into a 2 or 3 liter beaker, nearly fill the beaker with water and mix the contents thoroughly. Allow to stand five minutes and decant the supernatant liquid into a second beaker; refill the first with water and again mix the contents. After five minutes more, decant the second beaker into the third, the first into the second, refill, and again mix the first. Continue this operation, decanting from the third beaker into the sink, until the lighter material is washed out from the ketchup; then collect the sand from the three beakers in a tared Gooch crucible. Dry, ignite, and weigh. Your attention is especially called to the fact that under "sand" should only be reported the figure obtained by this method. The results obtained by the determination of ash insoluble in hydrochloric acid are not applicable to the determination of sand in tomato ketchup, partly because the percentage present is so small and the sand is so unevenly distributed that reliable results can only be obtained by taking a larger sample than is possible in the determination of ash.

Qualitative determinations.—Make qualitative examinations for benzoic acid, saccharin, boric acid, starch.

Report also the following.—Sugar-free, salt-free soluble solids; ratio of insoluble solids to sugar-free, salt-free soluble solids; ratio of nitrogen in lead acetate filtrate to total nitrogen; ratio of ammoniacal nitrogen to total nitrogen; ratio lactic acid to citric acid; ratio citric acid to sugar-free, salt-free soluble solids; ratio total protein to sugar-free, salt-free soluble solids.

Examine the original sample before mixing and report the presence and appearance of any sediment which may appear on the bottom.

ANALYTICAL RESULTS.

For purposes of investigation two samples of ketchup were prepared after a cookbook recipe; one was made from clean, sound, ripe tomatoes with stems and spots cut out, and the other was made from tomatoes which had been kept in the laboratory spread out on the bench until every one had started to decay—some were covered with a green mold. Owing to the very large amount of spices demanded by this recipe the two samples of ketchup tasted practically alike.

The sample made from sound tomatoes was designated "A," that from decayed tomatoes "B." Three pints of each of these were sent to four collaborators, who were requested to make a complete analysis according to the methods just given.

Reports were received from two of the collaborators and these, with the referee's own results, have been tabulated as follows:

Comparative percentage results by three analysts on two samples of ketchup.

Determinations.	"A" samples (sound tomatoes).			"B" samples (decayed tomatoes).		
	1	2	3	1	2	3
Total solids	23.80	23.69	22.90	18.93	19.21	19.67
Insoluble solids	2.39	2.38	2.25	1.83	1.80	1.88
Soluble solids	21.41	21.31	20.65	17.10	17.41	17.79
Ash	2.74	2.80	2.77	2.46	2.42	2.87
Alkalinity of ash (per cent potassium carbonate in salt-free ash)	56.8	60.3	43.6	54.6	72.0	25.7
Alkalinity of ash (cc tenth-normal acid in original ketchup)	.848	.900	.700	.871	1.0	.40
Sodium chlorid	1.71	1.77	1.66	1.34	1.46	1.24
Reducing sugars before inversion	13.10	13.92	13.76	8.00	8.39	8.78
Reducing sugars after inversion	14.70	15.56	15.36	11.95	12.52	12.67
Polarization after inversion (°V.)	31° C. −5.5	.... −6.2	30.5° C. −5.0	33.5° C. −4.2	.... −4.6	30° C. −4.0
Sucrose	1.50	1.56	1.52	3.75	3.92	3.50
Total acids, as citric	2.56	2.37	2.21	2.29	2.19	2.23
Fixed acids, as citric	1.05	1.17	.62	.63	.79	.68
Volatile acids, as acetic	1.43	1.15	1.40	1.58	1.33	1.45
Butyric acid	Present.	....	None.	Present.	....	None.
Formic acid	None.	....	Present.	None.	....	Present.
Lactic acid	.20	.39	.28	.559	.63	.32
Citric acid	.502	.480	.570	.354	.40	.510
Nitrogen	.338	....	.349	.269	....	.223
Protein (N×6.25)	2.11	....	2.18	1.68	....	1.39
Ammonia by distillation with magnesium oxid	.0612	.0580	.056	.0228	.019	.019
Nitrogen partition with basic lead acetate	.263	....	.268	.103	....	.137
Pectin determination	1.17	1.37	.97	.75	.91	.94
Sand	.0133	....	.026	.017	....	.008
Saccharin, qualitative	None.	....	None.	None.	....	None.
Boric acid, qualitative	None.	....	None.	None.	....	None.
Benzoic acid, qualitative	None.	....	None.	None.	....	None.
Starch, qualitative	None.	....	None.	None.	....	None.
Sugar-free, salt-free soluble solids	5.10	4.06	3.71	4.01	3.64	4.27
Ratio of insoluble solids to sugar-free, salt-free soluble solids	1:2.13	1:1.70	1:1.65	1:2.19	1:2.02	1:2.26
Ratio of nitrogen in basic lead acetate filtrate to total nitrogen	1:1.28	....	1:1.30	1:2.61	....	1:1.62
Ratio of ammoniacal nitrogen to total nitrogen	1:6.71	....	1:7.59	1:14.34	....	1:14.
Ratio lactic acid to citric acid	1:2.51	1:1.29	1:2.02	1:0.63	1:0.63	1:1.6
Ratio citric acid to sugar-free, salt-free soluble solids	1:10.10	1:8.46	1:6.51	1:11.32	1:9.10	1:8.37
Ratio of total protein to sugar-free, salt-free soluble solids	1:2.41	....	1:1.70	1:2.40	....	1:3.07
Sediment before opening	None.	....	None.	None.	....	None.

DISCUSSION OF RESULTS.

The agreement between the different analysts is fair, except in the determinations of alkalinity of ash, per cent of potassium carbonate in the salt-free ash, and of citric and lactic acids. The number of collaborators was not large enough to allow any definite conclusions to be drawn. The results, however, show the desirability of further work on the determination of lactic and citric acids. The work done in the Bureau of Chemistry by Bacon and Dunbar has demonstrated the value of the lactic-citric ratio in interpreting a ketchup analysis. It is of considerable importance that a method be worked out that will give reasonably consistent results in the hands of operators of ordinary skill.

I would suggest that the lactic-acid method be modified up to the point of the ether extraction in the following manner:

Make 100 grams of ketchup up to 500 cc with water, mix thoroughly, centrifuge; pour off 400 cc through a filter, add 10 cc of 20 per cent lead acetate solution, and make up to 500 cc. After centrifuging again 400 cc should be poured off through a filter and a slight excess of dilute sulphuric acid added. Settle precipitate with centrifugal and pour directly into evaporating dish. Wash the lead sulphate, precipitate once with about 50 cc of water, and filter into the evaporating dish. Evaporate on steam bath to volume desired for the extraction apparatus. Extract for 18 to 20 hours in a liquid extractor with washed ether. (Ether sufficiently pure for this purpose may be prepared by shaking out ordinary ether once with a sodium hydroxid solution and then 10 times with small quantities of water.) To the ether extract is added about 20 cc of water and the ether is evaporated. Care must be taken to remove all traces of ether. The water is added before evaporation because of the possible presence of traces of sulphuric acid which might otherwise char the lactic acid. Approximately 3 grams sodium hydroxid are then added to the water solution and 50 cc of a 1.5 per cent solution of potassium permanganate are added from a pipette. This mixture is heated on a water bath at 100° C. for one-half hour. At the end of that time, or before, if the color is not a decided blue-black or purple, but is green or colorless above the layer of brown precipitate, more standard permanganate in measured portions is added, until, after heating one-half hour on a boiling water bath, the color is a blue-black or purple. The oxidation is then complete. The hot solution is strongly acidified with dilute sulphuric acid, about 50 cc of 10 per cent sulphuric acid, and standard oxalic acid run in from a burette until the solution is decolorized. In this laboratory a 5 per cent solution of oxalic acid is used for that purpose. Any slight excess of oxalic acid is titrated back with the same standard permanganate solution. It should be understood that any standard permanganate and oxalic acid solutions may be used within reasonable limits of strength. In alkaline solution, the permanganate oxidizes the lactic acid quantitatively to oxalic acid according to the equation:

$$2C_3H_6O_3+10KMnO_4=2(COOH)_2+4H_2O+2CO_2+5MnO_2+5K_2MnO_4$$

Then in acid solution, the oxalic acid is further oxidized by the permanganate to carbon dioxid and water according to the equation:

$$5(COOH)_2+2KMnO_4+3H_2SO_4=10CO_2+8H_2O+K_2SO_4+2MnSO_4$$

Calculation: The total weight of permanganate used in the oxidation of the lactic acid is determined by subtracting the permanganate equivalent of the oxalic acid used from the total amount used. The weight of permanganate times 0.237 equals the weight of lactic acid.

Better results are obtained if the ether extract is run down to dryness, taken up with water, and filtered before adding the alkali and permanganate.

RECOMMENDATION.

It is recommended—

(1) That these methods or modifications of them be tried another year, if possible, securing a much larger number of collaborators.

(2) That some experiments be tried to ascertain to what extent the nitrogen ratios can be influenced by the spice content of ketchup.

(3) That, as the complete scheme of analysis as outlined is very long and takes about 16 hours of practically continuous attention, a special attempt be made to eliminate all the determinations not actually necessary for the judging of a ketchup.

REPORT ON COLORS.

By W. E. MATHEWSON, *Associate Referee.*

In the cooperative work on colors done last year two problems were considered—the identification of the permitted coal-tar colors when used in admixture with each other and the identification of certain other dyes that for one reason or another seemed to merit special study. This season an examination has been made of methods used for separating and identifying nonpermitted colors in admixtures.

Samples of six artificially colored food products were sent out, together with the statement of a procedure used in the New York laboratory for dealing with such mixtures. This consists in making the samples strongly acid with hydrochloric acid (about one-third the volume of concentrated acid being added) and shaking out with a few portions of amyl alcohol. The alcohol extracts are combined, then shaken with a number of small portions of hot water. The amyl alcohol is then diluted with gasoline, given a few more washings with water, and finally treated with very dilute caustic soda. In general the higher sulphonated dyes are found in the first strongly acid washings, those of a lower degree of sulphonation appearing later. If the original mixture contained basic colors, these are first removed by making alkaline and shaking with ether, the ether solution of the color bases being then fractionated in a similar way by washing with water and finally with dilute acid.

The colors used in preparing samples are given in the following table:

Samples for the identification of nonpermitted colors in admixtures.

Number.	Food product.	Coloring matters.	S. and J. numbers.
1	Imitation cordial	Bordeaux B	65
		Erythrosin	517
2	do	Tartrazin	94
		Crocein Orange	13
3	do	Light Green SF Yellowish	435
		Acid Yellow G	8
4	do	Rhodamin B	504
		Methyl Violet	451
5[1]	do	Indigo Carmin	692
		Amaranth	107
		Naphthol Yellow S	4
6	Salad oil	Sudan II	49
		Anilin Yellow	7

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[1] Examination of Sample No. 5 some six weeks after its preparation showed that the indigo carmin had entirely disappeared.

All these dyes are known to be frequently used in foods, and all, with one or two possible exceptions, have been found in admixture with other colors. Since most methods of separation are more or less troublesome with impure dyes, commercial products were used in preparing the samples, excepting the Crocein orange, acid yellow, and the oil-soluble colors, which were made in the laboratory. The acid yellow, however, was prepared in the usual way by sulphonating aminoazobenzene, and hence corresponded to No. 8 of the Schultz-Julius-Green tables, a mixture of sulphonated derivatives. The results obtained are given below:

Cooperative results on color mixtures.

Analyst.	Coloring matters reported.		
	Sample 1.	Sample 2.	Sample 3.
E. H. Grant, New Orleans, La.	Erythrosin	Crocein Orange	Light Green SF Yellowish.
	Amaranth	Tartrazin	Metanil Yellow.
C. S. Brinton, Philadelphia, Pa.	Bordeaux B	Crocein Orange	Light Green SF Yellowish.
	Erythrosin	Tartrazin	Acid Yellow G.
H. M. Loomis, Seattle, Wash.	Bordeaux B	Crocein Orange	Light Green SF Yellowish.
	Erythrosin	Tartrazin	Acid Yellow G.
W. A. Brannon, Washington, D. C.	Bordeaux B	Crocein Orange	Light Green SF Yellowish.
	Erythrosin	Tartrazin	Acid Yellow G.
A. M. Doyle, Washington, D. C.	Bordeaux B	Crocein Orange	Light Green SF Yellowish.
	Erythrosin	Tartrazin	Acid Yellow G.
	Rhodamin B (trace)		

Cooperative results on color mixtures—Continued.

Analyst.	Sample 4.	Sample 5.	Sample 6.
E. H. Grant, New Orleans, La.	Methylene Blue Rhodamin B	Naphthol Yellow S Amaranth	Sudan I. Butter Yellow (trace).
C. S. Brinton, Philadelphia, Pa.	Methyl Violet Rhodamin B	Naphthol Yellow S Amaranth Indigo Carmin	Sudan I. Anilin Yellow.
H. M. Loomis, Seattle, Wash.	Crystal Violet Rhodamin B	Naphthol Yellow S Amaranth Indigo Carmin	Sudan I. Anilin Yellow.
W. A. Brannon, Washington, D. C.	Methyl Violet Rhodamin B	Naphthol Yellow S Amaranth	Benzeneazo-α-naphthylamin.
G. M. Bartlett, Boston, Mass.	....	....	Similar to Anilin Yellow.
A. M. Doyle, Washington, D. C.	Methyl Violet Rhodamin B	Naphthol Yellow S Amaranth	Sudan I. Unidentified Yellow.

Details as to the methods of separation and identification used were not stated in most cases, but apparently immiscible solvents were usually employed for the separation and the dyes subsequently identified by their spot reactions when dyed on wool, etc., applying data given in United States Department of Agriculture Circular No. 63, Allen, and other sources.

C. S. Brinton used glacial acetic acid (Cornellison's method) for extracting the coloring matter from the oil in Sample No. 6. E. H. Grant also followed this procedure, but prefers to saponify the oil directly. W. A. Brannon saponified the oil, but found it difficult to obtain a clean ether solution of the coloring matter. G. M. Bartlett dissolved the oil in ether and shook out with strong hydrochloric acid.

H. M. Loomis suggests that the amyl alcohol or ether solution of the colors obtained on shaking out the original mixture should be washed with acid or alkali (as the case may be) to remove sugar, etc., before beginning the fractional shaking out with water.

As the permitted colors are so much more frequently used than others, any satisfactory general method for the treatment of unknown mixtures must require no great amount of manipulation or time when only these are present. Usually, however, the main object of the work is to determine whether or not nonpermitted dyes have been used, the identification of others being of interest only as bearing on this question. Such a method, therefore, should involve a minimum use of reagents likely to destroy or alter chemically any of the coloring matters and should be of such character that the deportment of the dye may be predicted with some accuracy from its constitution. Also it must be capable of being adapted to mixtures containing relatively small amounts of one component. These requirements seem best satisfied, on the whole, by the use of immiscible solvents.

Dichlorhydrin, suggested as a solvent by H. A. Seil, has been given some comparative study.[1] Of most dyes it extracts from a solution of given acidity much less than does amyl alcohol. However, it takes up far more Light Green SF Yellowish (and similar acid greens) than the latter solvent, and is useful for separating these after the other colors have been removed.

T. M. Price's recent paper[2] describing a separation of the permitted colors in dry mixtures gives new analytical data, but probably did not appear early enough to be made use of in the cooperative work.

It is suggested that the study of the separation of mixtures be continued by the association and that work bearing on the identification of the common natural coloring matters be begun.

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[1] U. S. Department of Agriculture, Bureau of Chemistry Cir. 89.

[2] U. S. Department of Agriculture, Bureau of Animal Industry Cir. 180.

REPORT ON VINEGAR.

By W. A. **Bender**, *Associate Referee.*

No cooperative work was done this year on vinegar, the referee's work having consisted in a revision of the provisional methods, which is recommended for the reason that new methods of importance have been in use for some time, and modifications have been made in some of the older methods. The referee considers it highly important to bring the provisional methods up to date with the least possible delay and a revision is therefore submitted for adoption and further study.

The methods were first written up substantially the same as those sent out last year by R. E. Doolittle with samples to the various Government laboratories, and copies were sent to representative food chemists throughout the country with a request for criticism. With these criticisms in mind the final copy was prepared. The methods as herein presented, except section 9, have been used for a year and a half in the New York and Washington laboratories of the Bureau of Chemistry, and were tested last year in conjunction with the referee's work and are mentioned in his report. (See Bul. 137, pp. 59, 60, and 61.) In case they are adopted the most important changes that will be effected in the methods as now printed in Bulletin 107, Revised, are as follows:

1. *Addition of the glycerin determination.*—This determination as worked out and tested by Ross in the case of wine and vinegar has been incorporated without change. Its accuracy has been further tested by both E. H. Goodnow and the referee, separately and together. It has been further shown by extensive work done in vinegar factories by the referee and Mr. Goodnow under the supervision of Messrs. Tolman and Doolittle that it is an essential determination for judging the purity of a vinegar. In Judge Willard's decision, at the recent vinegar trial in Minneapolis, it was held that the evidence showed this method to be accurate and essential.

2. *Reducing sugars direct after evaporation.*—It is a well-known fact that vinegar contains varying amounts of volatile reducing bodies, which are incorrectly estimated as sugar in the method formerly used. To get the true amount of sugar present it is, therefore, necessary to drive off this volatile matter by evaporation and use the residue for the determination. Clarification and neutralization are omitted following the referee's suggestion of last year.

3. *Fixed acid* is determined by titration of the residue obtained after repeated evaporation. The objection to the present method of Bulletin 107, Revised, is the difficulty of an accurate estimation of volatile acid by steam distillation.

4. *Estimation of color by the brewer's scale.*

5. *Determination of color removed by fuller's earth* is added for the reason that it is of value in the case of the addition of a large amount of caramel to a diluted vinegar.

6. *The alcohol precipitate* has been shown to be of value in detecting products made from decomposed pomace, waste, or apples. The referee believes that further cooperative work should be done on this method.

7. *The pentosan determination* is made for the same reason as stated in the preceding paragraph. This is a standard method which gives concordant results, and is extremely valuable in cases where material unfit for consumption has been used.

The following determinations given in Bulletin 107, Revised, have been omitted from the present revision:

12. Oxalic acid; 13 and 14, detection of bitartrate of potassium and free tartaric acid; 15 and 16, detection and determination of free mineral acids; 19, detection of dextrin; 20, detection of coloring matters, and 22, detection of

foreign pungent materials. The referee does not know that any recent work has been done along these lines and personally does not feel able to include them in the recommendations, but sees no objection to leaving them as they stand until question arises as to their value.

RECOMMENDATIONS.

It is recommended—

(1) That method 5 for glycerin by S. H. Ross be adopted provisionally, as published in the Proceedings for 1910. (Bul. 137. pp. 61–63.)

(2) That method 21 for pentosans be adopted provisionally. (This is the provisional method of Bulletin 107, Revised, p. 54, applied to vinegar with the direction for the use of the proper amount of hydrochloric acid to account for the water of the vinegar.)

(3) That the other new methods and modifications submitted be printed and studied further during the coming year.

METHODS FOR THE ANALYSIS OF VINEGAR.

1. *Preparation of sample.*—For microscopical examination employ the original sample, but for chemical analysis filter if turbid.

2. *Calculation of results.*—Express all results as grams per 100 cc.

3. *Specific gravity.*—Determine as directed under XIII, wine, page 83, Bulletin 107, Revised.

4. *Alcohol.*—Measure 100 cc of the sample into a round-bottom distilling flask. Make faintly alkaline with saturated caustic soda solution, add a small scrap of paraffin, distil almost 50 cc, make up to 50 cc at temperature of sample, filter if necessary, and determine specific gravity by pyknometer or Sprengel tube. Calculate per cent by volume, or grams per 100 cc, from Table II, page 203, bearing in mind that the alcohol strength of the distillate is twice that of the original vinegar.

5. *Glycerin.*—Ross's method. Bulletin 137, page 61.

6. *Solids.*—Measure 10 cc of filtered vinegar into a tared flat-bottom platinum dish of 50 mm diameter, evaporate on the water bath to a thick sirup and dry for exactly two and one-half hours in the drying oven at the temperature of boiling water; cool and weigh. It is essential to use a flat-bottom dish.

7. *Total reducing matters before inversion.*—Proceed according to Munson and Walker's method (Bul. 107, Rev., p. 241), using 10 or 20 cc of sample. Express results as grams of invert sugar per 100 cc. Malt vinegar should be clarified with sodium phosphotungstate.

8. *Reducing sugars before inversion, after evaporation.*—Evaporate 50 cc to 5 cc on the water bath. Add 25 cc water and evaporate to 5 cc. Again add 25 cc water and evaporate to 5 cc. Transfer to a volumetric flask, make up to the mark, and proceed as under "7," using a quantity equivalent to 10 or 20 cc of sample.

9. *Reducing sugar after inversion.*—Proceed as under "8." After the last evaporation to 5 cc transfer to a 100 cc flask with 70 cc of water and invert by one of the methods given under "VI. General methods" (*c*), page 40. Nearly neutralize with caustic soda, make up to the mark and proceed as under "7," using a quantity equivalent to 10 or 20 cc of sample.

10. *Polarization.*—If the lead precipitate is heavy add to 50 cc of sample 5 cc of lead subacetate solution, shake and let stand 30 minutes, filter and polarize, preferably in a 200 mm tube, correcting for dilution. If lead subacetate gives only a turbidity, add to the 50 cc sample in flask 10 cc alumina cream, shake, let stand 30 minutes, filter, and polarize, correcting for dilution.

11. *Ash.*—Measure 25 cc into a tared platinum dish, evaporate to dryness on the steam bath, heat in the muffle at low heat to expel inflammable gases, treat the charred portion with a few cubic centimeters of water and evaporate dry on the bath; replace in the muffle at low redness for 15 minutes, and continue the alternate evaporation and heating until a white or gray ash is obtained, at no time allowing the temperature to exceed a dull red; cool in desiccator and weigh.

12. *Solubility and alkalinity of soluble ash.*—Add to the above ash about 10 to 15 cc distilled water, bring to a boil, and filter through a 9 cm quantitative

filter. Repeat the operation twice; transfer the ash completely to the filter paper and wash with three successive portions of hot water; dry and ignite the filter with the undissolved residue at low red heat, cool, weigh, and calculate as insoluble ash. Cool the filtrate and titrate with tenth-normal hydrochloric acid, using methyl orange as indicator. Express results as number of cubic centimeters of tenth-normal hydrochloric acid per 100 cc sample.

13. *Soluble and insoluble phosphoric acid.*—Insert "8. Phosphoric acid of the ash," Bulletin 107, Revised, page 102.

14. *Total acids.*—Insert "9. Total acids," Bulletin 107, Revised, page 103.

15. *Fixed acid.*—Measure 10 cc into a 200 cc porcelain casserole, evaporate just to dryness, add 5 to 10 cc of water and again evaporate; repeat until at least five evaporations have taken place and no odor of acetic acid can be detected. Add nearly 200 cc of recently boiled distilled water and titrate with tenth-normal alkali, using phenolphthalein. One cubic centimeter of tenth-normal alkali is equivalent to 0.0067 gram of malic acid.

16. *Volatile acid.*—Calculate the fixed acid as acetic and deduct from the total acid. Express as acetic acid.

17. *Lead precipitate.*—To 10 cc in a test tube add 2 cc normal lead acetate (20 per cent solution), shake, and let stand one-half hour. Express as turbidity, light, medium, heavy, or very heavy.

18. *Color.—Brewer's scale.*—Read in good, reflected daylight, using 0.5-inch cell and the Lovibond scale.

19. *Color removed by fuller's earth.*—To 50 cc add 25 grams of fuller's earth, shake at intervals for 30 minutes and filter through a fluted filter. Put equal volumes of treated and original sample in adjoining colorimetric tubes, make to 50 cc with water, and compare the degrees of color in some form of colorimeter. Express results as per cent of color removed. On account of its varying quality it is necessary for the analyst to standardize the fuller's earth used against vinegars of known purity.

20. *Alcohol precipitate.*—Evaporate 100 cc of vinegar to about 15 cc. It has been observed that when there is considerable sugar in the vinegar if the sample is evaporated to too low a volume, on adding the alcohol a gummy or stringy precipitate occurs instead of a flocculent one. When the sugar content is high, therefore, the evaporation should not be carried beyond 20 cc. To this residue add 200 cc of 95 per cent alcohol slowly and with constant stirring, and allow the mixture to stand overnight. From this point follow the method for determination of alcohol precipitate under "XII. Fruits and fruit products," page 80, Bulletin 107, Revised.

21. *Pentosans.*—Follow the method as given under "VI. General methods," page 54, Bulletin 107, Revised. To 100 cc of vinegar add 43 cc of hydrochloric acid (specific gravity 1.19), and then distil exactly according to directions given.

22. *Heavy metals.*—Insert "23. Heavy metals," Bulletin 107, Revised, page 105.

23. *Detection of preservatives.*—Insert "24. Detection of preservatives," Bulletin 107, Revised, page 105.

REPORT ON FLAVORING EXTRACTS.

By R. S. HILTNER, *Associate Referee.*

PLAN OF WORK.

The plan for cooperative work on this subject for this year was based largely on the recommendations made by the referee and adopted by the association at the last meeting. These recommendations called for the study of the method of determining vanillin, coumarin, and normal lead number in one weighed portion, as proposed by Winton, Lott, and Berry, and the investigation of the Woodman-Newhall test for caramel in vanilla extracts; the study of the La Wall-Nelson tests for capsicum in ginger extracts and of the methods for the examination of nutmeg and wintergreen extracts.

In addition to these investigations that were recommended, it was thought advisable to study some of the more promising methods, bearing directly on these subjects, that have been published recently. These methods comprised, *first*, a test for caramel in vanilla extracts, using the method described by Tolman (Bul. 132, p. 90) to determine per cent of color insoluble in amyl alcohol (Marsh reagent); *second*, Denis's method for the detection of prune juice and sherry residues in vanilla extracts;[1] *third*, Mitchell's modification of the Seeker test for ginger in alcoholic solutions (see p. 137); *fourth*, Howard's modified general method for the determination of essential oils in alcoholic solutions[2] applied to nutmeg, wintergreen, and peppermint extracts.

An effort was made to prepare the samples in such a manner that the reliability of each of the methods, under varying conditions, could be ascertained. Sets of samples, with descriptions of the methods to be employed, were sent to the 15 members of the association who had offered to collaborate. More or less complete reports were received from the following chemists:

1. E. H. Berry, Chicago.
2. E. Bloomberg, Galveston.
3. C. S. Brinton, Philadelphia.
4. Courtney Conover, Pittsburgh.
5. Fred F. Flanders, Boston.
6. E. H. Grant, New Orleans.
7. A. W. Hanson, Kansas City.
8. A. M. Henry, Florida State Agricultural Department.
9. M. B. Kennedy, University of Nevada, Reno.
10. F. W. Liepsner, Kansas City.
11. Byron McClelland, New York.
12. E. L. P. Treuthardt, Washington.
13. Burton B. Wilcox, New York.
14. C. O. Dodge, Washington, D. C.
15. R. S. Hiltner, Denver, Colo.

To these chemists the associate referee desires to acknowledge his indebtedness for the data furnished and the interest manifested; also to A. L. Winton for valuable assistance and for the complete report from his laboratory. The names of the analysts have been arranged in alphabetical order and numbered accordingly. To simplify the presentation of the data in tabular form, the analysts will be referred to by number rather than by name.

VANILLA EXTRACTS.

Collaborators were asked to use the following methods in examining the seven samples that were furnished:

METHODS FOR EXAMINATION OF VANILLA EXTRACTS.

Determinations are to be made in one weighed portion of sample.

Special reagents.—Distilled water, boiled until free from carbon dioxid.

Standard lead acetate solution, 80 grams of C. P. crystallized lead acetate dissolved in water and made up to 1 liter.

Ammonium hydroxid solution, containing approximately 2 per cent of ammonia.

Hydrochloric acid solution, containing approximately 10 per cent of hydrochloric acid.

(*a*) *Vanillin and coumarin.*

(See Winton's paper, p. 147.)

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[1] J. Ind. Eng. Chem., 1911, 3: 254.

[2] J. Ind. Eng. Chem., 1911, 3: 252.

(*b*) *Normal lead number.*

(See Winton's paper, p. 148.)

(*c*) *Residual color after precipitation with lead acetate.*

(See Winton's paper, p. 148.)

(*d*) *Caramel.*

Test each sample for caramel by the Woodman and Newhall method. (See Cir. 66, p. 19.)

(*e*) *Color insoluble in amyl alcohol.*

(See Winton's paper, p. 149.)

(*f*) *Prune extract.*

Examine the samples for prune juice as directed by Denis (J. Ind. Eng. Chem., 1911, 3:254). Report results as positive or negative.

In each case express an opinion as to whether the sample is a genuine standard vanilla extract U. S. P. In samples that are found not standard state the nature of the adulterant indicated.

DESCRIPTION OF SAMPLES.

The following is a description of the samples that were prepared:

No. 1. Pure vanilla extract made according to the U. S. Pharmacopœia formula, from Bourbon, Seychelles, Comores, and Mexican beans (extract made at the U. S. Food and Drug Inspection Laboratory, Chicago).

No. 2. Vanilla extract (Mexican and Tahiti beans) adulterated with extract of dried prunes, synthetic vanillin, and colored with caramel.

No. 3. Pure vanilla extract, made according to the U. S. P. formula from a good grade of Mexican beans.

No. 4. A wholly factitious product, containing 25 per cent tonka-bean extract (10 per cent tonka beans, 20 per cent sugar, 70 per cent diluted alcohol) and 75 per cent of extract of dried prunes, to which was added enough synthetic vanillin to amount to 0.15 per cent in the finished product. Caramel was used to give the desired color.

No. 5. Pure vanilla extract, made according to the U. S. P. formula from a rather inferior grade of Tahiti beans.

No. 6. Contained about 40 per cent Tahiti-bean extract (No. 5) adulterated with synthetic vanillin so as to yield 0.17 per cent, and with other material to imitate genuine vanilla extract. Caramel was used for color.

No. 7. Commercial vanilla extract made in Denver and warranted by the manufacturer to be pure and according to U. S. standard, from Mexican beans 34 per cent, Bourbon 60 per cent, and Tahiti 6 per cent.

In preparing the factitious samples Nos. 2 and 6 an effort was made to imitate genuine vanilla extracts in the matter of color, vanillin content, lead number, and also to approximate the odor and flavor of the natural product. Sherry residues were not added to any of the samples. It seems inadvisable to make public the exact formulæ used. Members of the association who may be interested in the matter may, of course, secure the information by correspondence.

ANALYTICAL RESULTS.

The results as reported are given in Table 1.

TABLE 1.—*Cooperative work on vanilla extracts.*

Analyst and sample number.	Vanillin.	Coumarin.	Normal lead number.	Residual color in filtrate after precipitation with lead acetate.				Color insoluble in Marsh's amyl alcohol reagent.	Caramel, Woodman-Newhall method.	Denis method.		Conclusions as to character of samples.
				Red.	Yellow.	Total color, red and yellow.	Total color, brewer's scale.			Prune juice.	Sherry residue.	
Sample 1.												
	Per cent.	*Per cent.*		*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*				
1	0.19	None.	0.57	4.0	6.9	6.1		23.2	Pos	Neg	Neg	**Appears to be straight vanilla extract.**
2	.20	None.	.46				9.9		Neg	do	do	
3									do	do	do	Probably pure extract.
4	.17	None.	.59				7.4	19.0	Doubtful	do	do	Genuine vanilla extract.
6	.19	None.	.54				5.7	40.0	Pos	do	Pos	Artificially colored with **caramel and sherry residue.**
8	.17	None.	.59						Neg	do	do	Vanilla extract colored **with sherry** residue.
9	[1].10	[2]0.03	.63									
10	[1].21	[2].02	.56						Neg			
11	.19	None.	.53				5.2	16.7	do	Neg	Neg	**U. S. P. extract.**
12			.52									
14	.14	None.	.56	3.0	6.0			23.0	Neg			**Straight extract.**
15	.18	None.	.52	4.0	5.5	5.1	5.7	19.3	do	Neg	Neg	**Denis test indicates genuine vanilla.**
Sample 2.												
1	.17	None.	.42	9.6	14.0	12.8		52.6	Pos	Pos	Neg	**Vanillin solution, caramel, and prune juice.**
2	.17	None.	.34				16.9		do	do	do	
3									Doubtful	do	do	**Probably vanilla extract and prune juice.**
4	.15	None.					15.0	54.0	Pos	do	do	**Caramel and prune juice indicated.**
6	.16	None.	.35				14.8	50.0	do	do	do	**Vanillin, caramel, and prune juice added.**
8	.18	None.	.45						Neg	do	do	**Vanilla extract colored with prune juice.**
9	[1].10	[2]0.01	.46									
10	[1].13	[2].02	.21						Pos			
11	.17	None.	.37				10.9	59.5	do	Doubtful	Neg	**Apparently U. S. P. with caramel.**
12			.39									
14	.14	None.	[3].44	11.0	18.0			55.0	Pos			**Straight; reenforced.**
15	.16	None.	.42	9.1	16.2	14.3	11.6	63.1	do	Pos	Neg	**Denis test indicates caramel and prune extract.**
Sample 3.												
1	.20	None.	.51	3.7	6.2	5.5		23.2	Pos	Neg	Neg	**Appears straight vanilla extract.**

2	.35	None.	.72				8.3		Neg	do	do	
3									do	do	Pos	Probably vanilla extract and sherry residue.
4	.30	None.	.83				6.2	23.0	do	Pos	Neg	
6	.27	None.	.63				5.9	35.6	do	Neg	Pos	Genuine extract with sherry residue.
8			.68									
9	[1]1.15	[2]0.01	.81									
10	[1].32	[2].02	.72						Neg			
11	.22	None.	.55				5.1	15.1	do	Neg	Neg	U. S. P.
12			.75									
14	.22	None.	[3].56	5.0	7.0			15.0	Neg	Pos	Neg	Large amount of prune; little, if any, vanilla.
15	.32	None.	.77	4.1	4.8	4.6	7.0	22.2	Doubtful	Neg	Doubtful	Denis test gave no positive indications.
Sample 4.												
1	.14	0.040	.19	19.5	35.5	30.1		90.9	Pos	Pos	Neg	Tonka extract or coumarin, caramel, and prune juice.
2	.15	.087	.16				56.8		do	do	do	
3									do	do	do	Imitation; caramel and prune juice.
4	.13	.020	.21				34.6	85.0	do	do	do	Caramel and prune juice indicated.
6	.14	.034	.13				36.0	45.0	Neg	do	do	Artificial; prune juice and possibly caramel.
8	.17	.032	.23						do	do	do	Vanilla extract, coumarin, and prune juice.
9	[1].07	[2].021	.30									
10	.16	.034	.18						Pos			
11	.14	.040	.20				25.2	82.0	do	Pos	Neg	Artificial; possibly prune juice added.
12			.20									
14	.16	.030	.20	26.0	47.0			75.0	Pos	Neg	Pos	Sherry; little, if any, real vanilla.
15	.14	.034	.20	22.0	43.1	36.7	24.3	85.2	do	Pos	Neg	Denis test indicates prune extract and caramel.
Sample 5.												
1	.19	None.	.85	3.5	6.3	5.6		25.0	Pos	Neg	Neg	Appears straight vanilla extract.
2	.20	None.	.72				9.7		Neg	do	do	
3									do	do	Pos	Probably vanilla extract and sherry residue.
4	.17	None.	.86				7.4	20.0	do	Doubtful	Doubtful	
6	.20	None.	.83				6.7	33.8	do	Pos		Colored with prune extract.
8	.20	None.	1.00						do	Neg	Pos	Vanilla extract, vanillin, and sherry residue.
9	[1]1.02	[2]0.015	.95									
10	[1]1.19	[2].026	.81						Neg			
11	.19	None.	.83				6.1	20.3	do	Doubtful	Doubtful	Apparently U. S. P. with coloring matter.
12			.86									
14	.11	None.	.92	4.0	6.0			21.0	Neg			Very poor imitation vanilla.
15	.18	None.	.85	4.0	5.7	5.3	6.8	19.8	Doubtful	Pos	Neg	Denis test indicates prune extract.

[1] Not purified.

[2] Not identified.

[3] At 42° C.

TABLE 1.—*Cooperative work on vanilla extracts*—Continued.

Analyst and sample number.	Vanillin.	Coumarin.	Normal lead number.	Residual color in filtrate after precipitation with lead acetate.				Color insoluble in Marsh's amyl alcohol reagent.	Caramel, Woodman-Newhall method.	Denis method.		Conclusions as to character of samples.
				Red.	Yellow.	Total color, red and yellow.	Total color, brewer's scale.			Prune juice.	Sherry residue.	
Sample 6.	*Per cent.*	*Per cent.*		*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*				
1	.16	None.	0.39	15.1	20.2	18.8		76.9	Pos.	Neg.	Neg.	Vanillin solution and caramel.
2	.16	None.	.31				26.0		do.	do.		
3									Neg.	do.	Neg.	Not standard vanilla; probably artificially colored.
4	.15	None.	.40				19.3	70.0	Pos.	Pos.	do.	Caramel and prune juice indicated.
8									do.	Neg.	do.	Vanilla extract colored with caramel.
9	[1].08	[2]0.013	.47									
10	[1].20	[2].008	.38						Pos.			
11	.16	None.	.37				14.4	59.0	do.	Neg.	Neg.	Apparently U. S. P. with caramel.
12			.35									
14	.13	None.	.37	15.0	24.0			51.0	Neg.	Pos.	Pos.	Prunes and sherry added.
15	.15	None.	.38	13.9	22.1	29.8	17.5	60.2	Pos.	Doubtful.	Doubtful.	Denis test gave no positive indications.
Sample 7.												
1	.30	None.	.73	3.3	5.4	4.8		26.3	Pos.	Neg.	Neg.	Appears true extract but for high vanillin.
2	.30	None.	.60				8.0		Neg.	do.	do.	
3									Doubtful.	do.	do.	Highly colored dilute vanilla extract.
4	.23	None.	.78				6.6	23.0	do.	Pos.	do.	
6	.36	None.	.71				5.5	42.5	Neg.	do.	do.	Contains vanillin and prune extract.
8	.32	None.	.89						do.	Neg.	Pos.	Vanilla extract, vanillin and sherry residue.
9	[1].15	[2]0.041	.77									
10	[1].31	[2].016	.74						Neg.			
11	.31	None.	.66				5.1	24.5	do.	Neg.	Neg.	Contains added vanillin; not U. S. P.
12			.72									
14	.25	None.	.74	3.0	3.0			24.0	Neg.			Prune; vanilla may be present.
15	.31	None.	.70	2.7	4.2	4.0	5.3	25.7	Doubtful.	Neg.	Neg.	Denis test indicates genuine vanilla extract.

[1] Not purified.

[2] Not identified.

Winton has observed that in vanilla extracts the determination of the percentages of colors (red and yellow) of the original extract remaining in the filtrate after precipitation with lead acetate affords valuable information for distinguishing genuine extracts from artificially colored and otherwise adulterated products. The results reported by three analysts are given in Table 1. Further studies have shown Winton that the ratio of red to yellow in the extract and in the filtrate furnishes a still better criterion. The amounts of red and yellow that comprise the composite color of the solution may be determined quite exactly with a Lovibond tintometer, using the standard color glasses. Although it was requested that this method be tried, only three analysts reported on the subject. It seems that very few of the laboratories are equipped with the necessary apparatus, particularly with complete sets of the standard color glasses. In Table 2 are recorded the results of the tests of this method:

TABLE 2.—*Comparison of color of vanilla extracts and filtrates.*

Analyst and sample numbers.	Color value.				Ratio of red to yellow.	
	Original extract.		Lead acetate filtrate.			
	Red.	Yellow.	Red.	Yellow.	Extract.	Filtrate.
Sample 1.						
1	35	100	1.4	6.9	1:2.9	1:4.9
14	30	113	1.1	7.0	1:3.8	1:6.5
15	35	113	1.4	6.2	1:3.2	1:4.4
Sample 2.						
1	50	125	4.8	17.6	1:2.5	1:3.7
14	46	170	5.3	32.0	1:3.7	1:6.0
15	48	123	4.4	20.0	1:2.5	1:4.5
Sample 3.						
1	38	113	1.4	7.0	1:3.0	1:5.0
14	29	123	1.5	8.3	1:4.2	1:5.5
15	58	258	2.4	12.4	1:4.4	1:5.1
Sample 4.						
1	43	85	8.4	30.2	1:2.0	1:3.6
14	31	83	7.9	39.0	1:2.7	1:4.9
15	39	89	8.6	38.4	1:2.3	1:4.4
Sample 5.						
1	40	110	1.4	7.0	1:2.8	1:5.0
14	28	122	1.2	7.0	1:4.3	1:5.8
15	37	125	1.5	7.2	1:3.4	1:4.8
Sample 6.						
1	33	85	5.0	17.2	1:2.6	1:3.4
14	29	90	4.3	22.0	1:3.1	1:5.1
15	33	85	4.6	18.8	1:2.6	1:4.0
Sample 7.						
1	48	133	1.6	7.2	1:2.8	1:4.5
14	40	182	1.1	5.8	1:4.5	1:5.8
15	48	160	1.3	7.0	1:3.2	1:5.4

As no inch or half-inch cell was available a 50 mm polariscope tube was used. This gave too much color. Readings were reduced to 1-inch cell.

ABSTRACT OF COMMENTS OF ANALYSTS.

E. H. Berry: The Woodman-Newhall test for caramel was tried on three uncolored authentic samples of vanilla and gave positive results on all. In official samples 1, 3, and 5, the Denis test indicated sherry residues. An authentic extract made from Mexican beans gave a similar result, namely, a small amount of light-colored precipitate insoluble in acetic acid. Pure extracts of Bourbon and Tahiti beans gave lead precipitates which dissolved almost completely in the dilute acetic acid reagent.

A. L. Winton: Samples 1, 3, and 5 appear to be straight vanilla extract, and the only thing that leads me to suspect sample No. 7 is the high vanillin. It may be true vanilla extract to which vanillin has been added, although I have known true extracts to go as high as 0.3 per cent, or even, in very exceptional cases, 0.35 per cent. Samples 2 and 6 appear to contain the same substances with the exception of prune juice, which was found to be present in 2, but not in 6. The other constituents, other than vanilla extract, are evidently vanillin solution and caramel, although the evidence of added vanillin is not absolutely conclusive. The samples might be straight vanilla extracts of high vanillin content, merely diluted. This is not, however, probable. Sample No. 4, in addition to vanilla extract, contains either tonka extract or coumarin solution, caramel, and prune juice. In reaching these conclusions I have paid no attention to Woodman and Newhall's test, which we have not found valuable.

The Marsh test appears to corroborate the results obtained on the color values of the extract and of the lead filtrate as followed by us. It has the advantage of direct comparison of the two liquids in a colorimeter, thus eliminating the cumbersome figures for red and yellow required in the other process. On the other hand, it has the disadvantage that another portion of the extract must be taken. Both methods give ambiguous results on two samples of extract made from Ceylon beans in this laboratory. These results appear to be abnormal. In fact, the results in color value and Marsh test are about the same as for your sample No. 2, which appears to contain caramel. These two extracts have perplexed us quite a deal and I have about reached the conclusion that either the beans have been extracted (they contain only 0.07 per cent vanillin) and soaked, say, in caramel solution, or else they represent a very abnormal product, comparable, for example, with cow's milk containing only 2 per cent of fat.

E. Bloomberg: In each case the residue was obtained for coumarin, but only those cases in which a qualitative test showed its presence were reported. The prune juice test seems inconclusive. In every case, excepting No. 6, a residue was obtained after the addition of acetic acid. In Nos. 1 and 7 the residue was yellowish and granular. In Nos. 3 and 5 a considerable yellowish flocculent residue remained. This would seem to indicate the presence of sherry wine coloring.

C. S. Brinton: In the experience of this laboratory (Philadelphia) we do not consider the modified paraldehyde test for caramel entirely satisfactory, unless a dark-brown precipitate, which adheres to the test tube, is obtained. In samples 1, 2, 6, and 7 small amounts of brown flocculent nonadherent precipitate were obtained, and in all four of these samples I believe that caramel is absent. The Denis test for prune juice and sherry residue has been found unreliable. Four pure extracts made according to the U. S. P. formula from Bourbon, Mexican, and South American beans and Mexican "cuts" when subjected to this test gave very inconsistent results. My conclusions as to the character of the seven official samples were based largely on the results of the Woodman-Newhall and Denis tests and must be considered accordingly.

Courtney Conover: Sample No. 1 appears to be a genuine vanilla extract. The other six samples seem abnormal in certain respects. Nos. 2, 3, 4, 6, and 7 respond positively to the test for prune extract. No. 5 gave a somewhat doubtful test for sherry residue. The lead numbers of samples 3, 5, and 7 are very high and apparently confirm the results of the Denis test. The reactions for prune extract, to an inexperienced person, would not be very definite. The Woodman-Newhall test for caramel is somewhat unsatisfactory, except in the presence of large amounts. The improved lead subacetate method for caramel, proposed by Albrech and Conover, gave positive evidence of the presence of caramel in Nos. 2, 4, and 6. Nos. 1, 3, and 5 appeared to be abnormal extracts, possibly colored with a very small amount of caramel or other color. No. 7 seems to contain no caramel.

E. H. Grant: Results indicate that No. 1 is wholly artificial, colored with caramel and sherry residue. Likewise No. 4, except that prune extract is present instead of sherry. In No. 5 the excessively high lead number indicates the addition of foreign substances. It is colored with prune extract. No. 7 is probably fortified with vanillin and colored with prune extract.

F. W. Liepsner: The figures reported for per cent vanillin and coumarin represent the weights of the dried but unpurified ether extracts. The residues weighed as coumarin were not tested qualitatively for that substance. The results therefore must be judged accordingly.

A. M. Henry obtained better results by measuring out at the beginning enough 10 per cent hydrochloric acid into the separatory funnels to neutralize the ammoniacal solutions of vanillin. This gives very little opportunity for the ammonia to attack the vanillin.

M. B. Kennedy: The figures reported under vanillin and coumarin represent the unpurified, dried residues from the ether extractions. Qualitative tests for coumarin were not made.

DISCUSSION BY THE REFEREE.

For the determination of vanillin, lead number, etc., I would recommend that 50 cc of the sample be used instead of 50 grams, for the reason that the United States standard for vanilla extract requires at least 10 grams of vanilla beans per 100 cc and because the U. S. P. and other formulas specify a certain weight of beans for unit volume of menstruum. Furthermore the specific gravity of solvents used in commercial extracts varies so decidedly that it would seem advisable to measure rather than weigh the sample and obtain results in terms of grams per 100 cc, and thus determine more directly the matter of conformity to the standard.

To insure exact aliquots of the solution for determining vanillin and coumarin and lead number, as directed in paragraphs (*a*) and (*b*), it would seem desirable to specify in the text of the method, first, that after adding lead acetate the solution should be warmed to approximately 38° and made up to the mark with water at the same temperature; and, second, that the aliquot portions of the filtrate should be drawn off while the solution is at the incubator temperature. These details would lead to uniformity of results.

The color of the filtrates, after precipitation with lead acetate, by the Winton-Lott-Berry method, usually gives dependable information as to the character of the extracts. In genuine products, irrespective of the nature of the solvent, the color of the filtrate is light straw tint and will seldom exceed 10 units of the brewer's scale, as gauged in a 1-inch cell of a Lovibond tintometer. Except with very artfully adulterated samples this very simple test will suffice to distinguish pure extracts from those artificially colored, especially with caramel.

It seems to be the almost universal experience of analysts that residues of vanillin and coumarin, resulting from the evaporation of ether extracts, are seldom, if ever, pure, but are contaminated with more or less gummy matter. Consequently results from weighing the crude product are usually too high. The error will often amount to 0.04 per cent. If the residues are extracted with petroleum ether by the method suggested and the vanillin and coumarin thus purified, the results are frequently too low, because the resinous or gummy matter, being insoluble in petroleum ether, often prevents complete solution of the vanillin and coumarin, with which it is intimately mixed. At best, the method of purification as prescribed, especially for vanillin, is tedious and exacting and requires skillful manipulation to prevent loss. The results reported for No. 3 emphasize the difficulties the analysts had in determining vanillin. That sample yielded about 0.04 per cent of gasoline-insoluble matter in the crude vanillin residue. To offset these difficulties, the following procedure is suggested:

Draw off the ether extract of vanillin into a dry 100-cc beaker, evaporate the ether, and dry the residue over sulphuric acid as usual; weigh the beaker and contents gross, then heat in an oven at 105° C. for three or four hours, or until constant weight is attained. Finally cool and weigh. Loss in weight indicates vanillin. Follow the same plan with the ether extract of coumarin. If it be desired to determine the purity of the volatile matter, the beaker may be heated cautiously, first on a hot plate and the sublimate condensed on a cool cover glass and afterward identified by melting points and qualitative tests. The beaker and residue should then be placed in the oven to complete the volatilization. Vanillin and coumarin appear to be completely volatile at 105°, and the gummy matter seems not to suffer change at that temperature. From various kinds of pure vanilla extracts it exhibits uniformly the same characteristics, viz, brown color, insolubility in water and petroleum ether, ready solubility in alcohol and in alkalis, and slight solubility in ether. In view of these peculiarities of solubility, it would seem to be advantageous to use washed ether to extract the vanillin and coumarin from the samples.

Having had little experience with the Woodman-Newhall test for caramel as applied to vanilla extracts, I was unable to distinguish clearly between the artificially colored and pure extracts by this method. Samples 3, 5, and 7, containing no caramel, gave doubtful results. The test with phenylhydrazin seems entirely unreliable.

The Denis test was not very satisfactory. It responded quite positively with the samples containing prune extract, but with samples containing none the results were not strikingly negative, especially with No. 5, which was pure extract made from Tahiti beans. This gave positive reactions for prune juice. I think with practice the analyst would be able to use this method to advantage in many cases.

CONCLUSIONS.

The results as received from the collaborators seem to warrant the following conclusions with reference to vanilla extracts:

(1) That the Winton-Lott-Berry method can be fully relied upon for the determination of vanillin and coumarin when proper precautions are taken to purify the extracted matter.

(2) That the lead number obtained by the use of normal or neutral lead acetate gives a more dependable number or constant than by sub- or basic acetate of lead, for the reason assigned by the authors of the method.

(3) That the percentage of residual color (either total color or component yellow and red tints) in the lead filtrates, as determined by the Winton-Berry method, gives valuable information as to the presence or absence of added color in vanilla extracts. The principal thing now lacking is a schedule of limits for this percentage of color in pure extracts from various kinds of beans. This also applies to the subject of normal lead number.

(4) That the Tolman method for determining color insoluble in amyl alcohol (Marsh reagent) also gives trustworthy information regarding the coloring matter of vanilla extracts. Data for establishing limiting figures are needed here also. It appears possible that it will be necessary for each analyst to prepare his own standards for comparison. The "personal equation" figures largely in these methods involving color values and color comparison.

(5) That the Woodman-Newhall test for caramel in vanilla extracts can not be relied upon for positive results. The method would be of value only for confirmatory tests and for distinguishing caramel from other artificial coloring in the hands of those who have had considerable experience with various grades of extracts.

(6) That the Denis method for detecting prune juice and sherry residue in vanilla extracts is likewise unreliable. Only one analyst reported correctly regarding the character of the seven samples submitted, and he has had much experience lately with vanilla extracts. The results of the Denis test were considered in connection with the other data. This fact forces the conclusion that the Denis method is sound, but that it can be relied upon only by those who keep in practice and have had considerable experience.

GINGER EXTRACTS.

METHODS AND SAMPLES.

Four samples labeled "Ginger extract" were forwarded to the analysts, with descriptions of the methods to be followed in testing for ginger and capsicum. The methods proposed were—

(*a*) Test each sample for ginger as directed in Circular 66, page 22. Also try Mitchell's modification of the Seeker test as follows:

Evaporate the ether extract as in the Seeker method. Add to the residue 10 or 12 drops of concentrated sulphuric acid and about 5 mg of vanillin. Mix thoroughly by rubbing with a glass rod; then allow a few drops of water to flow down into the mixture from the side of the dish. A persistent dark blue color indicates ginger.

Report results as positive or negative.

(*b*) Test the samples for capsicum by the La Wall-Nelson method (Circular 66, page 22), observing the precautions suggested by Doyle to prevent loss of capsicum by volatilization, namely, render the sample slightly alkaline with sodium hydroxid before evaporating the alcohol and then slightly acidify with sulphuric acid before the first extraction with ether. The final ether extract of the saponified solution should be washed with water until neutral to litmus and then allowed to evaporate spontaneously.

Report results as positive or negative.

It was especially desired to learn the value of Mitchell's modification of Seeker's test for ginger and to determine whether the details suggested by Miss Doyle would render more sensitive and positive the La Wall-Nelson test for capsicum.

Sample No. 1 was pure standard ginger extract, made according to the U. S. Pharmacopœia formula for tincture of ginger, using 20 grams of powdered ginger (Japan and Cochin) per 100 cc.

Sample No. 2 consisted of U. S. P. ginger extract (made from pulverized Japan ginger), 50 per cent; tincture of capsicum U. S. P., 0.5 per cent; alcohol, 49.5 per cent; colored with caramel.

Sample No. 3 was also adulterated. It contained less ginger and more capsicum (2.5 times as much) than No. 2. Sirup and caramel were added to approximate the total solids and color of standard ginger extract.

Sample No. 4 contained no ginger extract. It consisted of 2.5 per cent U. S. P. tincture of capsicum, mixed with sirup and caramel to approximate the total solids and color of standard ginger extract.

ANALYTICAL RESULTS.

The results as reported by the analysts are given in Table 3.

TABLE 3.—*Cooperative results on ginger extracts.*

Analyst and sample.	Ginger.		Capsicum.	
	Seeker method.	Mitchell method.	La Wall-Doyle method.	Nelson method.
Sample 1.				
1	Positive	Positive	Negative	Negative.
2	do	do	do	
3	do	do	do	Do.
4	do	do	do	Positive.
6	do	do	do	
7	do	do	do	Negative.
8	do	do	do	Do.
9	do		do	
11	do	Positive	do	Do.
12	do	do	do	
14	do	do	do	Do.
15	do	do	do	Do.

TABLE 3.—*Cooperative results on ginger extracts*—Continued.

Analyst and sample.	Ginger.		Capsicum.	
	Seeker method.	Mitchell method.	La Wall-Doyle method.	Nelson method.
Sample 2.				
1	Positive	Positive	Negative	Negative.
2	do	do	Positive	
3	do	do	do	Positive.
4	do	do	Negative	Negative.
6	do	do	Positive	
7	do	do	do	Do.
8	do	do	do	Do.
9	do		Negative	
11	do	Positive	Positive	Positive.
12	do	do	do	
14	do	do	do	Do.
15	do	do	do	Do.
Sample 3.				
1	Positive	Positive	Positive	Negative.
2	do	do	do	
3	do	do	do	Positive.
4	do	do	Negative	Negative.
6	do	do	Positive	
7	do	do	do	Positive.
8	do	do	do	Do.
9	do		Negative	
11	do	Positive	Positive	Do.
12	do	do	do	
14	do	do	do	Do.
15	do	do	do	Do.
Sample 4.				
1	Negative	Negative	Positive	Negative.
2	do	do	do	
3	do	do	do	Positive.
4	do	do	do	Negative.
6	do	do	do	
7	do	Positive	do	Positive.
8	do	Negative	do	Do.
9	do		Negative	
11	Doubtful	Positive	Positive	Do.
12	Negative	Negative	do	
14	Doubtful	Positive	do	Do.
15	Negative	Negative	do	Do.

ABSTRACTS OF COMMENTS OF ANALYSTS.

E. Bloomberg: By using Mitchell's method for ginger, allowing the concentrated sulphuric acid, after mixing, to flow onto the side of the dish and then adding a few drops of water to the thin layer of acid mixture, the blue color appears immediately.

C. S. Brinton found that the Mitchell test gave more strikingly positive results than the original Seeker method and therefore recommends that the modified method be adopted by the association. He regards the La Wall-Doyle method for capsicum as more reliable than the Nelson test.

C. Conover: Seeker's test gave better color reactions than Mitchell's modification. The tests for capsicum by the two proposed methods were inconsistent in samples 1 and 4. In No. 4 the La Wall-Doyle test was strongly positive, while, by the Nelson method, capsicum appeared to be absent.

E. H. Grant: With Mitchell's method I found it advisable to mix the residue with sulphuric acid and vanillin by rotating the dish, rather than by rubbing with a glass rod, as the amount of extracted matter is so high as to give a very dark color if the whole of the residue is taken up.

M. B. Kennedy tested the four ginger extract samples for capsicum by the proposed methods but obtained no positive results.

Byron McClelland: The Nelson test for capsicum appears to be not so decisive as the La Wall-Doyle.

E. L. P. Treuthardt: The Mitchell test for ginger gave a more pronounced coloration than the original method. Sample 4 gave a distinct blue color with the former, but only a faint violet tint, which was not regarded as a positive test, with the Seeker method. For the detection of capsicum the use of manganese dioxid more effectually decomposes the ginger, but it lessens the delicacy of the test for capsicum. The Doyle modification of La Wall's method gives a more positive indication of capsicum than does the Nelson test. In every case where capsicum was reported present, there was a distinct "bite" on the tongue surely due to capsicum. In all the samples there was sufficient ginger left in the capsicum residue to give a positive test by the Seeker-Mitchell method. The samples were evidently made up as follows: No. 1, straight ginger extract; No. 2, ginger extract with trace of capsicum and containing less ginger than No. 1; No. 3, weak ginger extract with slight amount of capsicum; No. 4, trace of ginger and much capsicum.

C. O. Dodge considers the color developed by the Seeker test for ginger to be better described as purple and regards the Mitchell modification as more sensitive than the original method.

DISCUSSION BY REFEREE.

I regard Mitchell's test for ginger as a little more sensitive but not so reliable as the Seeker method. Ether extracts of certain other spices were found to give, by the former method, color reactions (shades of blue and purple) that might be easily confused with the color due to ginger. For example, sample No. 4, which contained no ginger, gave a doubtfully positive test with concentrated acid, but a distinctly negative result with more dilute acid (Seeker method). Other analysts experienced the same difficulty in judging this sample. (See Table 3.) I observed that the purplish color developed in No. 4 by the Mitchell test was very fugitive, and in this respect could be clearly distinguished from reactions of ginger.

As a general rule the odor of the sample, especially after the alcohol has evaporated, is sufficient proof of the presence or absence of ginger.

The details of the test for capsicum, as proposed by Doyle, greatly improve the La Wall method. The test may be made a little more sensitive by allowing the final ether extract to drip slowly from the separatory funnel into a small watch glass and regulating the flow in accord with the evaporation, so as to have only a few drops of liquid in the dish at a time, thus restricting the residue to a small area. Determine taste by holding the residue in the watch glass against the tip of the tongue for about a half minute. A hot, stinging sensation distinguishes capsicum and is not easily confused with the mild, warm taste due to traces of ginger.

My experience was in accord with Mr. Treuthardt's with samples 1, 2, and 3, namely, that the residue to be tasted for capsicum (La Wall-Doyle test) gave positive test for ginger by the Seeker method, indicating that conclusions as to traces of capsicum must not be hastily drawn.

Nelson's modification proved unsatisfactory.

NUTMEG EXTRACTS.

The reliability of two methods for determining the percentage of oil of nutmeg in alcoholic solution was tested this year: (A) The A. O. A. C. method for lemon oil in extracts, as given in Bulletin 107, Revised, page 160. Since oil of nutmeg appears to be insoluble in dilute alcohol, and since it is of lower specific gravity than water, this method seemed to be directly applicable. (B) Howard's method as published in J. Ind. Eng. Chem., 1911, 3: 252. Following is an outline of the method:

Mix 20 cc of the sample in a separatory funnel with 50 cc of water and 2 drops of hydrochloric acid. Extract the separated oil with three portions of ether and transfer the extract, after washing once with water, to a Babcock milk flask. Rapidly evaporate the ether by immersing the flask, previously connected to an exhaust pump, in hot water, shaking at first gently and then violently. Finally, float the residual oil on cold water, whirl in a centrifuge, and measure. It was suggested that a thermometer of small diameter be inserted in the neck of the Babcock flask when expelling the ether to control the evaporation and prevent loss of oil. A sharp rise of temperature indicates complete expulsion of ether vapor.[1]

Two samples were forwarded to each analyst. Sample No. 1 contained 1.5 per cent and No. 2, 2.5 per cent by volume of oil of nutmeg.

In Table 4 are given the results as reported:

TABLE 4.—*Cooperative results in nutmeg oil in nutmeg extracts.*

Analyst.	Sample No. 1.		Sample No. 2.	
	Method A.	Method B.	Method A.	Method B.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
1	0.7	0.8	1.7	1.8
2	.6	1.1	1.4	2.1
3	1.0	1.9	1.9	[1]2.9
4	.6	.8	1.4	2.0
6	.7	1.0	1.7	1.8
7	.8	.7	1.6	1.6
8	.9	....	1.8	1.6
12	1.0	1.4	2.0	2.6
13	1.1	1.6	2.0	2.6
14	....	1.3	....	....
15	1.0	.8	1.8	1.6

Three determinations reported, namely, 3.95, 2.90, and 2.85.

ABSTRACTS OF COMMENTS OF ANALYSTS.

E. Bloomberg: Method A gives low results. An attempt was made to separate more of the oil by cooling the precipitated oil and then centrifuging. In this way No. 1 gave 1 per cent of oil and No. 2, 1.7 per cent. The precipitated oil was allowed to remain overnight at room temperature in the flask, securely corked, and then centrifuged. This method gave 1.0 per cent for No. 1 and 1.9 per cent for No. 2. Howard's method seems to offer several chances for loss of oil, owing to the solubility of the oil in the dilute alcohol, and also to the solubility of ether in the dilute alcohol, which, doubtless, carries some oil with it.

C. Conover: The use of a thermometer to control the evaporation in the Howard method seems unnecessary, the rise of temperature being immediately noticeable by the warming up of the Babcock bottle in the hand.

B. B. Wilcox: Results by Method A are too low, because of the solubility of the oil in dilute alcohol. By using 10 cc of the extract instead of 20 cc this difficulty was in some measure removed. The effect of the error in reading is, of course, doubled. It seems probable that by using a Babcock bottle of 100 cc or more capacity, and using 20 cc of the sample, results of some accuracy could be obtained. Repeated tests of Howard's methods with extracts of known strengths failed to give the true amounts of oil or concordant results. The difficulty seemed to be in some cases that the ether was not completely removed and in others that some oil was volatilized.

E. L. P. Treuthardt: In methods such as these, where the volume of the separated oil is measured, it is important that points at which the column of oil is to be read should be clearly stated. My experience indicates that the extreme points should always be the ones noted.

C. O. Dodge: The alcohol of these samples is very much stronger than is necessary to hold the oil in solution. Method A could be applied only after a preliminary dilution and the multiplication of errors renders the results valueless.

REFEREE'S DISCUSSION AND CONCLUSIONS.

I was unable to obtain either accurate or concordant results with the methods proposed or, in fact, with any modifications that suggested themselves. The principal difficulty experienced with the Howard method was that part of the extracted oil, after evaporating the ether, collected in small globules on the walls of the bottles and resisted every effort to dislodge them by mechanical means. My observations accord exactly with those noted by Mr. Wilcox.

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[1] Method offered by C. O. Dodge, Bureau of Chemistry, Washington, D. C.

Considering the results and comments of the 11 analysts who reported on these samples, it is apparent that the methods tried this year for determining oil of nutmeg in alcoholic solutions are of no value. Moreover, it appears that simple modifications of the methods fail to give reliable results. No analyst reported correctly the amount of oil present. The difficulty with Howard's method, as Mr. Wilcox states, seems to be that either the oil is partly volatilized giving low results or the ether is not completely removed giving high results.

As noted by Mr. Dodge, the alcohol used in preparing the samples of nutmeg, wintergreen, and peppermint extracts was of unnecessarily high proof. This strong alcohol was used for the purpose of testing the reliability of the method under extreme conditions.

WINTERGREEN EXTRACTS.

METHODS AND ANALYTICAL RESULTS.

Three methods were proposed for determining the percentage of oil of wintergreen in alcoholic solutions:

(*A*) Howard's method, (J. Ind. Eng. Chem., 1911, 3: p. 252), using cold sulphuric acid (1:2) for the floating medium. This method is the same as for oil in nutmeg extracts, as described above, except that dilute sulphuric acid is used to float the oil owing to the high specific gravity of oil of wintergreen.

(*B*) A modification of Hortvet and West's method.

Method B.—Pipette 50 cc of the sample into a separatory funnel, add 5 drops concentrated hydrochloric acid and 125 cc cold water. Shake out with three portions of ether, 30 cc, 20 cc, and 10 cc, respectively; wash with ether-saturated water as directed by Howard (supra); then transfer the ether extract to a Hortvet tube (as used for lead precipitate in maple products). Evaporate the bulk of the ether by aspirating cold through a two-hole cork, using one hole for a vent. Immerse the tube in boiling water and proceed according to Howard's method until the ether is eliminated. Finally add 15 cc hot water, centrifuge and measure the oil at room temperature.

This is a modification of Hortvet and West's method[1] as regards the apparatus used. It appeared feasible to use in this way the well-known Hortvet (lead precipitate) tubes, and thus obviate the necessity of special apparatus.

(*C*) Hortvet and West's method of saponifying the oil and weighing as salicylic acid. (J. Ind. Eng. Chem., 1909, 1:90.) The following is an abstract of this method:

Mix 10 cc of the extract with 10 cc of potassium hydroxid solution (10 per cent). Heat on the steam bath until the volume is reduced about one-half. Add a distinct excess of dilute hydrochloric acid; cool and extract with three portions of ether, 40 cc, 30 cc, and 20 cc, respectively. Filter the extract through a dry filter into a weighed dish, wash with 10 cc ether and allow to evaporate spontaneously. Dry over sulphuric acid in a desiccator, weigh, and calculate by the formula: Weight salicylic acid × 9.33 = per cent oil of wintergreen by volume.

Two samples were prepared. Sample No. 1 contained 2.5 per cent, and No. 2, 3.0 per cent of oil of wintergreen (natural) dissolved in 95 per cent alcohol. The results are given in Table 5.

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[1] J. Ind. Eng. Chem. 1909, 1: 90.

TABLE 5.—*Cooperative results on oil of wintergreen in wintergreen extracts.*

Analyst.	Sample No. 1.			Sample No. 2.		
	Method A.	Method B.	Method C.	Method A.	Method B.	Method C.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
1	2.5	2.6	2.48	3.2	3.2	2.95
2	2.9	3.0	....	3.4	3.3	....
3	[1] 2.9	....	....	[1] 4.2	....	....
4	2.3	2.6	2.50	2.7	3.2	3.00
5	3.8	3.6	2.53	3.7	3.8	2.97
6	2.5	....	2.47	3.0	....	2.98
7	2.4	2.5	2.39	3.1	3.2	2.98
8	2.8	2.2	....	5.4	3.0	....
12	2.5	2.6	3.50	3.1	3.1	4.20
13	....	....	2.64	....	....	3.24
14	2.5	....	2.49	3.0	....	2.99
15	2.5	2.2	2.46	3.0	2.8	2.98

[1] Analyst No. 3 reported sample No. 1, 2.85 and 6.25 and sample No. 2, 4.2 and 5.2.

ABSTRACTS OF COMMENTS OF ANALYSTS.

E. H. Berry: In Method B it was very difficult to remove all the ether.

C. Conover considers Method C, by saponification, to be very satisfactory.

B. B. Wilcox: The observations concerning Howard's method for nutmeg oil apply also to the determinations of oil of wintergreen and peppermint. Method B being similar to Howard's method was not tried.

C. O. Dodge: In Method B the evaporation from the Hortvet tube is extremely difficult owing to spurting. With Method C, I found that drying one hour in a desiccator, as specified, was not sufficient with the large amount of salicylic acid present.

R. S. Hiltner: Methods A and C proved very satisfactory in every particular. With Method A loss of oil by volatilization does not occur. Method B seems to have no special merit.

CONCLUSIONS.

For determining oil of wintergreen in alcoholic solutions, both the Howard method of extracting with ether and floating the extracted oil on dilute sulphuric acid and the Hortvet-West method of saponification of the oil and weighing as salicylic acid, gave satisfactory results, except in two or three cases. It would seem advisable to have both methods adopted by the association for counterchecking results. Method B failed to give accurate results and, having no special points of merit, need not be further considered by the association.

PEPPERMINT EXTRACTS.

METHODS AND RESULTS.

Cooperation was asked on two methods for the determination of oil of peppermint in alcoholic solutions:

(A) The same procedure as for lemon oil in lemon extract as given in Bulletin 107, Revised, page 160.

(B) Howard's method (J. Ind. Eng. Chem., 1911, 3: p. 252) observing the precaution suggested by Dodge to prevent evaporation of the oil, and using salt solution instead of cold water to float the oil. This method is the same as for nutmeg extracts (see p. 139), and appeared to be the most promising of any that had been proposed. It is a slight modification by the author of the original method, which the association adopted provisionally last year. The use of chloroform is omitted.

Two samples of peppermint extract were sent to the analysts. Sample No. 1 contained 2.5 per cent and sample No. 2, 3.25 per cent by volume of oil of peppermint (American), dissolved in alcohol. The results as reported are given in Table 6.

TABLE 6.—*Cooperative results on oil of peppermint in peppermint extracts.*

Analyst.	Sample No. 1.		Sample No. 2.	
	Method A.	Method B.	Method A.	Method B.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
1	0.5	2.4	0.8	3.4
2	.8	3.4	1.1	4.9
3	.8	3.5	1.6	5.1
4	.5	2.5	.9	3.8
5	.2	7.1	.8	6.8
7	.5	2.4	1.2	3.2
8	.6	2.4	.9	....
12	.8	2.6	1.2	3.7
13	.5	2.0	1.1	2.7
14	2.1	3.1	....	....
15	.8	2.4	1.5	3.0

ABSTRACTS OF COMMENTS OF ANALYSTS.

E. H. Berry: Method A has been generally recognized as valueless for use with peppermint extracts. The oil does not all precipitate.

E. Bloomberg: My comments on the methods for nutmeg apply with even greater force to the determination of oil of peppermint, because it is more soluble in dilute alcohol than oil of nutmeg. The same variations were tried with peppermint. On cooling and centrifuging No. 1 gave 1.4 and No. 2, 2.2 per cent. On allowing to stand overnight No. 1 gave 1.5 and No. 2, 2.6 per cent of oil.

E. H. Grant: Results on peppermint extract were very unsatisfactory. The exact difficulty is not apparent. With the association method the oil seemed to be quite soluble in the diluted sample. No. 1 yielded 2.9 per cent oil by Howard's method.

B. B. Wilcox: By using 10 cc of the sample instead of 22 cc in Method A, I obtained 2 and 2.7 per cent of oil, respectively, in Nos. 1 and 2.

CONCLUSIONS.

The methods tried this year for the determination of oil of peppermint are not to be relied upon. The precipitation method, corresponding to the association method for lemon oil, proved utterly worthless. Howard's method gave better results, but was far from satisfactory. A comparison of the percentages of oil present in the samples with the amounts reported in Table 6 will show how unreliable the methods proved.

RECOMMENDATIONS.

Summing up the work of the year, it appears that considerable progress has been made. The methods now at hand will enable the analyst to distinguish with certainty pure vanilla extracts. It may not be possible in every case to determine the exact composition of a factitious sample, but it will be feasible to report positively that such samples are adulterated.

Almost as much may be said of the methods for ginger. There is need, however, of a reliable method for the examination of ginger solids.

There is evident necessity also for better methods for determining essential oils in alcoholic solutions. This is especially true of oil of peppermint. "Extracts," "essences," and "tinctures" of peppermint, containing such varying amounts of oil in alcohol of high proof and low, are important articles of commerce and demand the attention of the food inspection chemist. Reliable methods are desired in this connection to check the growing practice of substituting oil from which menthol has been partially abstracted.

The two methods that have been proposed for the determination of oil of wintergreen in alcohol solutions seem worthy of adoption by the association without further study.

It is recommended—

(1) That the method of determining vanillin, coumarin, normal lead number, and residual color in one weighed portion, as proposed by Winton, Lott, and Berry, be provisionally adopted. In harmony with this recommendation, it is necessary to ask that recommendation No. 2 as adopted last year (Bul. 137, p. 120) be set aside and that the text of the method as given on page 128 (modified as suggested below) be substituted for all material under the heading No. 4, in Bulletin 107, Revised, pages 156 to 158, "4. Determination of vanillin, coumarin, normal lead number, and residual color." These determinations are all to be made in one weighed portion.

On account of the importance of this method as a means of distinguishing between genuine and imitation vanilla extracts, and in view of numerous changes of detail of procedure that have been suggested and that seem worthy of consideration, I ask that a committee, with Dr. Winton as chairman, be appointed at the forthcoming meeting of the association to consider the data of the analysts' reports of this year and the suggestions that have been made, and to draft a text for the method that will be fully in accord with the latest investigations of the subject. The findings of this committee should be accepted by the association as final for the provisional method for determining vanillin, coumarin, lead number, and residual color. In this way much valuable time and work may be saved.

(2) That the above method for determining vanillin, coumarin, normal lead number, and residual color in filtrate, in one weighed portion of sample, be further studied next year for the special purpose of ascertaining the limits of composition of standard vanilla extracts.

(3) That Tolman's method for determining per cent of color insoluble in amyl alcohol (Marsh reagent) be adopted as provisional, and that the text of the method as published in the Proceedings of the association (Bul. 132, p. 90) be inserted as (c) under "11. Test for coloring matter," in Methods of Analysis (Bul. 107, Rev., p. 159). It is further recommended that this method be studied next year for the purpose of determining the range of values for pure vanilla extracts.

(4) That the methods for the determination of benzaldehyde in almond extract be further studied, with the view of determining the reliability of the methods and also the conditions under which aldehyde is oxidized to benzoic acid in commercial extracts, as well as the extent of such oxidation.

(5) That Mitchell's modification of the Seeker test for ginger be adopted as provisional and added to paragraph 3 under "Ginger extract," as an optional or confirmatory test. The description of this test is as follows:

Extract the dealcoholized sample and evaporate the ether as in the Seeker method. Add to the residue 10 or 12 drops of concentrated sulphuric acid and about 5 mg of vanillin. Mix thoroughly by rubbing with a glass rod; then allow a few drops of water to flow down into the mixture from the side of the dish. An azure blue color indicates ginger.

(6) That the method for the detection of capsicum in ginger extract, as proposed by Miss Doyle, modifying the La Wall method, be adopted as provisional. The Doyle method is not essentially different from La Wall's. The details of procedure, however, are such as to make the test more positive and are set forth more clearly than in the latter method. The following is a condensed statement of the method:

To 10 cc of the extract cautiously add dilute sodium hydroxid until the solution reacts very slightly alkaline with litmus paper. Evaporate at about 70° C. to about one-quarter of the original volume, render slightly acid with dilute sulphuric acid, testing with litmus paper. Transfer to a separatory funnel, rinsing the evaporating dish with water, and extract with an equal volume of ether. Avoid emulsification, shaking the funnel gently for a minute or two. Draw off the lower layer and wash the ether extract once with about 10 cc of water. Transfer the washed ether extract to a small evaporating dish, render decidedly alkaline with alcoholic KOH, and evaporate at about 70° until the residue is pasty; then add about 20 cc more of N/2 alcoholic potash and allow to stand on a steam bath until the gingerol is completely saponified. Usually about one-half hour is required. Dissolve the residue in a little water and transfer with water to a small separatory funnel. The volume should not exceed 50 cc. Extract the alkaline solution with an equal volume of ether. Wash the ether extract repeatedly with small amounts of water until no alkaline reaction with litmus is given. Transfer the washed extract to a small evaporating dish, allowing the ether to evaporate spontaneously. Finally, test the residue for capsicum by moistening the tip of the finger, rubbing it around on the bottom and sides of the dish and then applying the finger to the end of the tongue. A hot, stinging or prickly sensation, which persists for several minutes, indicates capsicum.

(7) That the Street-Morrison method (Bul. 137, p. 76) and other available methods for examining and identifying the components of the total solids of ginger extracts be a subject for study next year. Such a method is necessary for proving adulteration in alcoholic extracts of ginger.

(8) That the subject of the determination of oil of nutmeg in nutmeg extract be studied further, and that other methods be tried next year. Both of the methods tried this year proved utterly unreliable.

(9) That, for the determination of oil of wintergreen in wintergreen extracts, both of the following methods be adopted provisionally.

First, Howard's method as described in J. Ind. Eng. Chem., 1911, 3:252, using cold dilute sulphuric acid (1:2) for the floating medium.

Second, Hortvet and West's method of saponifying the oil and weighing as salicylic acid (J. Ind. Eng. Chem., 1909, 1:90).

By the first method the volume of the extracted oil is measured in the stem of a Babcock flask.

By the second method the amount of oil present is found by determining the principal ingredient, viz, methyl salicylate, which constitutes about 99 per cent of oil of wintergreen. Since these methods are based on entirely different principles, they afford a valuable means of checking results.

(10) That the Howard method for the determination of oil of peppermint in alcoholic solutions, which was adopted provisionally last year, be abandoned, and that the subject be given further study. The original method proposed by Howard, which was adopted last year (Bul. 137, p. 76), was modified, in the interests of greater accuracy, by its author, and the new method published in J. Ind. Eng. Chem., 1911, 3:252. The new method was tried this year by eight collaborating chemists. The results reported were quite unsatisfactory, lacking both accuracy and uniformity.

THE CHEMICAL COMPOSITION OF AUTHENTIC VANILLA EXTRACTS, TOGETHER WITH ANALYTICAL METHODS AND LIMITS OF CONSTANTS.

By A. L. Winton and E. H. Berry.

Variation in Composition of Vanilla Extract.

Standard vanilla extract, as recognized by the food analyst, the pharmacist, and the reputable manufacturer, is a definite product in that it contains in 100 cc of the solution the alcohol-soluble materials from 10 grams of vanilla beans; it is indefinite in that it is more or less variable in its composition, owing partly to variations in the percentages of moisture and soluble constituents of the bean and partly to differences in the methods of extraction. The quality of the extract is also variable, being dependent on the variety and grade of bean, but with our present knowledge this can not be definitely correlated with differences in composition.

The purpose of this paper is to show the variation in composition of extracts made in the laboratory from different varieties, grades, and lengths of vanilla beans and by the use of different menstrua, thus furnishing data to aid in distinguishing between the genuine product and imitations. The methods of analysis, some of which have been developed by the writers, are also described in detail.

Varieties of Vanilla Beans.

The vanilla bean is a native of Mexico, from which country the finest grades are still obtained. Mexican vanilla is largely consumed in the United States. The product chiefly used in Europe is collectively known as Bourbon vanilla, which includes true Bourbon (the product of Réunion), Seychelles, Comores, Madagascar, etc. Vanilla of somewhat less commercial value and of relatively small importance is produced in South America, Java, and Ceylon. The vanilla of Tahiti has long been recognized as of inferior flavoring value and has commanded a correspondingly low price.

The following quotations per pound, which obtained at the time the samples for this investigation were purchased (August, 1909), show the relative commercial value of the several varieties: Mexican, $2.50 to $5; Bourbon (including Comores, Seychelles, and Madagascar), $2.50 to $3.25; South American, $2.50 to $3; Java, $2.50; Ceylon, $2.40; Tahiti, $0.80.

Vanillons, also known as pompona or La Guayra vanilla, being the fruit of a different species (*Vanilla pompona*) is not true vanilla, and an extract prepared from it is not vanilla extract. These beans are distinguished from genuine vanilla by their shorter length and greater breadth. They are used chiefly in the preparation of sachet powder.

Most of the commercial varieties of beans can be distinguished only by an expert, as the differences are slight and hardly describable. Tahiti beans, however, have in the past been strikingly different from the others, owing to their high moisture content and greasy appearance, and extracts prepared from these beans have been characterized by their light color and characteristic inferior flavor. These defects, however, will doubtless be largely obviated owing to the enforcement of a law which went into effect in French Oceania during the present year, prohibiting the exportation of beans which have not been officially inspected and passed by a board of examiners.

The Tonka bean, which is a true bean in that it is the seed of a legume, as distinguished from the so-called vanilla bean, which is the fruit of an orchid, has little in common with the latter. The extract from this bean contains coumarin but no vanillin, and has a low color value and low normal lead number.

PROCESSES OF MANUFACTURE OF VANILLA EXTRACT.

The U. S. P. process, which is regarded as standard, is not generally used by manufacturers of vanilla extracts other than pharmacists. Many consider that 50 or even 45 per cent alcohol is quite as efficient as the 60 per cent of the U. S. P. formula, and some substitute glycerin for sugar, for the reason that it is said to retain the flavor better in cooking. The use of alcohol of greater dilution than 45 per cent is impracticable, because it extracts from the beans gelatinous constituents which render percolation difficult or impossible. Alcohol alone, without either sugar or glycerin, is an efficient solvent, but the product is not as salable, owing to the prejudice of the consumer, who is accustomed to the sweet taste.

The time of extraction and of percolation varies in different factories. Most manufacturers soak their beans with alcohol for days and even months before percolation. The U. S. P. process is probably the least efficient of all the commercial processes unless care is taken to use for the menstruum alcohol which has been previously macerated with or percolated through the residue from the extraction of a previous lot of beans; consequently, tentative standards of composition based on the analyses of U. S. P. extracts are not unfavorable to the manufacturer who might employ a more complete method of extraction. The coloring matter of the bean, which is highly prized in the extract, is particularly slow of extraction.

METHODS FOR THE ANALYSIS OF VANILLA EXTRACT.

DETERMINATION OF VANILLIN AND COUMARIN.

Modified Hess and Prescott method.[1]—Pipette 50 cc of the extract directly into a tared 250 cc beaker with marks showing volumes of 80 and 50 cc; dilute to 80 cc, and evaporate to 50 cc in a water bath kept at 70° C. Dilute again to 80 cc with water and evaporate to 50 cc. Transfer to a 100 cc flask, rinsing the beaker with hot water, add 25 cc of standard lead acetate solution (80 grams of chemically pure crystallized lead acetate, made up to 1 liter), make up to the mark with water, shake and allow to stand 18 hours at a temperature of from 37 to 40° C. in a bacteriological incubator, in a water bath provided with a thermostat, or in any other suitable apparatus. Filter through a small dry filter and pipette off 50 cc of the filtrate into a separatory funnel.

If a determination of normal lead number is desired, pipette off 10 cc of the filtrate into a beaker and proceed as described on page 148. In the latter case, the water used throughout the process should be boiled until free from carbon dioxid. If coloring with caramel is suspected, determine the color value of the original extract and the filtrate (page 148).

To the 50 cc of the filtrate in the separatory funnel add 20 cc of ether and shake. Draw off carefully the aqueous liquid, together with any ether emulsion, and then remove the clear ether solution to another separatory funnel. Repeat the shaking of the aqueous liquid with ether three times, using 15 cc each time.

Shake the combined ether solutions four or five times with 2 per cent ammonium hydroxid, using 10 cc for the first shaking and 5 cc for each subsequent shaking. In drawing off the ammoniacal solution, care should be taken not to allow any of the ether solution to pass through with it. Reserve the ammoniacal solution for the determination of vanillin.

Transfer the ether solution to a weighed dish and allow the ether to evaporate at room temperature. Dry in a sulphuric acid desiccator and weigh. If the residue is pure coumarin, it should have a melting point of 67° C., respond to the Leach test, and be completely soluble in three or four portions of petroleum ether (boiling point 30° to 40° C.), stirring with each portion 15 minutes.

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[1] J. Amer. Chem. Soc., 1899, 21: 256; ibid., 1902, 24: 1128; ibid., 1905, 27: 719; U. S. Dept. Agr., Bureau of Chemistry Bul. 132, p. 109, and Bul. 137, p. 120.

Add to the ammoniacal solution 10 per cent hydrochloric acid to slightly acid reaction. This should be done without delay, as the ammoniacal solution on standing grows slowly darker with a loss of vanillin. Cool, and shake out in a separatory funnel with four portions of ether, as described for the first ether extraction. Evaporate the ether solution at room temperature in a weighed dish, dry over sulphuric acid, and weigh. The residue should be pure vanillin, free from any appreciable amount of color and with a melting point of 80° C.

DETERMINATION OF NORMAL LEAD NUMBER.

Winton and Lott method.[1]—Mix the 10 cc aliquot of the filtrate from the lead acetate precipitate obtained in the determination of vanillin and coumarin (page 147), with 25 cc of water, boiled until free from carbon dioxid, and a moderate excess of sulphuric acid. Add 100 cc of 95 per cent alcohol and mix again. Let stand overnight, filter on a Gooch crucible, wash with 95 per cent alcohol, dry at a moderate heat, ignite at low redness for three minutes, taking care to avoid the reducing flame, and weigh. The normal lead number is calculated by the following formula:

$$P=\frac{100\times0.6831(S-W)}{5}=13.662(S-W)$$

in which P=normal lead number, S=grams of lead sulphate corresponding to 2.5 cc of the standard lead acetate solution as determined in blank analyses, and W=grams of lead sulphate obtained in 10 cc of the filtrate from the lead acetate precipitate as above described.

The standard of the lead acetate solution as determined by blank analyses does not change appreciably on standing; it should, however, be checked from time to time, especially if the bottle is opened frequently, thus permitting absorption of carbon dioxid.

In all steps of the process only water free from carbon dioxid should be used.

DETERMINATION OF COLOR VALUE OF THE EXTRACT.

Pipette 2 cc of the extract into a 50 cc graduated flask and make up to the mark with a mixture of equal parts of 95 per cent alcohol and water. Determine the color value of this diluted extract in terms of red and yellow by means of a Lovibond tintometer, using the 1-inch cell. To obtain the color value of the original extract, multiply the figures for each color by 25.

For example, a reading of 0.6 red and 2.1 yellow obtained on the diluted extract corresponds to a color value of 15 red and 52 yellow calculated to the original extract.

DETERMINATION OF RESIDUAL COLOR AFTER PRECIPITATION WITH LEAD ACETATE.

Determine the color value, in terms of red and yellow, of the filtrate from the lead acetate precipitate, obtained in the determination of "vanillin and coumarin" (p. 147), using the 1-inch Lovibond cell. Multiply the reading by 2, thus reducing the results to the basis of the original extract.

In case the actual reading of the solution is greater than 5 red and 15 yellow, as may happen if the extract is highly colored with caramel, the one-half or one-quarter inch cell should be employed and the readings multiplied respectively by 4 or by 8.

Divide the figures for red and yellow respectively by the corresponding figures of the original extract and multiply the quotients by 100, thus obtaining the percentages of the two colors remaining in the lead acetate filtrate.

For example, if the color value of the original extract is 15 red and 52 yellow and the color value of the lead acetate filtrate, also measured in the 1-inch cell, is 0.6 red and 2.4 yellow, then the residual color after precipitation with lead acetate calculated to the basis of the original extract is 1.2 red and 4.8 yellow or 8 per cent of the red and 9.2 per cent of the yellow.

Calculate also the ratio of red to yellow in both extract and lead filtrate.

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[1] U. S. Dept. Agr., Bureau of Chemistry Bul. 132, p. 110, and Bul. 137, p. 120.

DETERMINATION OF COLOR INSOLUBLE IN AMYL ALCOHOL.[1]

Marsh test.—Evaporate 25 cc of the extract until the odor of alcohol is no longer apparent and the liquid is reduced to a thick sirup. Dissolve the residue in water and alcohol, using 26.3 cc of 95 per cent alcohol, and making up to volume in a 50 cc flask with water. Transfer 25 cc of this solution to a separatory funnel; add 25 cc of the Marsh reagent and shake, not too vigorously, to avoid emulsification. Allow the layers to separate and repeat the shaking twice more. After the layers have separated clearly, run off the lower layer into a 25 cc cylinder, and make up to volume with 50 per cent by volume alcohol. Filter if necessary and compare in a colorimeter with the remaining 25 cc portion (which has not been extracted with the reagent) and express the results as per cent of color insoluble in amyl alcohol.

The Marsh reagent is prepared as follows:

Mix 100 cc of amyl alcohol, 3 cc of sirupy phosphoric acid, and 3 cc of water; shake before using. If the reagent becomes colored on standing, the amyl alcohol should be redistilled over 5 per cent phosphoric acid.

U. S. P. VANILLA EXTRACTS MADE IN THE LABORATORY.

In order to learn the influence of variety, grade, and length of bean on the composition of vanilla extract, 74 samples were obtained from one of the leading importers. The number of grades of each variety represented were as follows: Mexican, 5; Bourbon, 5; Seychelles, 3; Madagascar, 3; Comores, 4; South American, 3; Ceylon, 1; Java, 1; Tahiti, 1. Whenever possible, two or three lengths of each grade were secured and separate extracts made of each. In addition, one sample each of Mexican cuts and splits, also one of Bourbon splits, were included. One sample of Vanillons and two of Angostura Tonka beans, one "prime" and the other "bloaters," were secured.

Determinations of moisture were made only in the case of one sample of Tahiti beans, which was found to contain 50.53 per cent. Probably none of the samples contained less than 25 per cent of moisture, and none, except the Tahiti samples, as high as 50 per cent. As regards this point they represented fairly the commercial product, which is stated not to change appreciably in moisture content during storage in the tin boxes used for the purpose.

PREPARATION OF EXTRACTS.

The method employed was that for *tinctura vanillæ* (tincture of vanilla) as described in the United States Pharmacopœia, eighth revision, page 484, as follows:

> Vanilla, cut into small pieces and bruised, 100 grams; sugar, in coarse powder, 200 grams; and alcohol and water, each a sufficient quantity to make 1,000 cc.
>
> Mix 650 cc of alcohol with 350 cc of water. Macerate the vanilla in 500 cc of the mixture for 12 hours; then drain off the liquid and set it aside. Transfer the vanilla to a mortar, beat it with the sugar into a uniform powder, then pack it in a percolator and pour upon it the reserved liquid. When this has disappeared from the surface, continue the percolation by gradually pouring on sufficient menstruum to make 1,000 cc of tincture.

In all cases, wherever the quantity of the material would permit, 200 grams of the beans and proportionate quantities of the other ingredients were employed. The vanilla, preparatory to extraction, was ground as finely as possible in a meat chopper. The maceration of the ground vanilla was carried on, as directed, for 12 hours, while the percolation, after grinding the residue with the sugar, consumed approximately 12 additional hours.

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[1] The method is that employed by Tolman and Hillyer (Bul. 122, p. 206; Bul. 132, p. 90) for the detection of caramel in whisky. The application of this process to the examination of vanilla extract was first suggested by Hiltner.

EXTRACTS FROM RESIDUES OF FIRST EXTRACTION.

The residues from the U. S. P. extractions, representing 200 grams of the beans, were macerated with 500 cc of 60 per cent (by volume) alcohol for five months in glass-stoppered bottles. At the end of that time the contents of the bottle were transferred to a percolator, and after the liquid had run through, fresh portions of 60 per cent alcohol were added until the percolate measured 1 liter.

This liter of second extract accordingly corresponded to the 2 liters of the first extract, but in calculating the analytical results, all of the figures were reduced to the same basis as that of the first extract.

The total percentage of vanillin in the bean may be accurately obtained by adding the figures obtained in the two extracts and multiplying by 10. The totals for normal lead number and color value may also be calculated, but these figures are less significant, as they represent merely what was obtained by the solvents and methods of extraction employed.

RESULTS OF ANALYSES.

In Table 1 are given the detailed results of the analyses of the U. S. P. extracts, together with the maximum, minimum, and average results of the analyses of the extracts made from each variety of beans, as well as of all of the analyses, excluding Ceylon vanilla, Vanillons, and Tonka beans.

The detailed results of the analyses of the extracts prepared from the residues from the U. S. P. extracts, together with the maximum, minimum, and average figures, excluding the varieties noted in the last paragraph, appear in Table 2.

TABLE 1.—*Analyses of vanilla extracts (tincture of vanilla U. S. P.) made in the laboratory.*

Serial No.	Kind and quality of bean.	Length of bean.	Vanillin.	Normal lead number.	Color value.				Per cent of total color in lead filtrate.		Ratio of red to yellow.		Per cent of total color insoluble in amyl alcohol.
					Extract (total color).		Lead filtrate.[1]						
					Red.	Yellow.	Red.	Yellow.	Red.	Yellow.	Extract.	Lead filtrate.	
	Mexican:	*cm.*	*Gms. per 100 cc.*						*Per cent.*	*Per cent.*			*Per cent.*
1	First	22	0.17	0.52	22	62	1.2	4.8	5	8	1:2.8	1:4.0	19.5
2	Do	17	.16	.47	19	55	1.2	4.8	6	9	2.9	4.0	23.9
3	Second	22	.17	.56	25	80	1.4	7.0	6	9	3.2	5.0	21.3
4	Do	19	.17	.55	25	72	1.0	4.8	4	7	2.9	4.8	19.5
5	Do	15	.17	.61	29	75	1.2	5.4	4	7	2.6	4.5	20.5
6	Third	23	.19	.58	27	102	1.4	7.8	5	8	3.8	5.6	22.7
7	Do	19	.19	.59	27	97	1.4	6.2	5	6	3.6	4.4	24.4
8	Do	15	.20	.50	30	97	1.4	6.2	5	6	3.2	4.4	21.1
9	Third (splits)	21	.19	.62	50	135	1.8	7.8	4	6	2.7	4.3	20.4
10	Fourth	20	.19	.64	35	102	1.4	6.8	4	7	2.9	4.9	20.4
11	Fifth	22	.15	.66	35	115	1.8	7.6	5	7	3.3	4.2	20.8
12	Do	16	.16	.68	37	120	1.8	7.8	5	7	3.2	4.3	22.2
13	Cuts	....	.16	.62	56	154	2.0	8.0	4	5	2.8	4.0	19.0
	Maximum	23	.20	.68	56	154	2.0	8.0	6	9	3.8	5.6	24.4
	Minimum	15	.15	.47	19	55	1.0	4.8	4	5	2.6	4.0	19.0
	Average	**19**	**.17**	**.58**	**32**	**97**	**1.5**	**6.5**	**5**	**7**	**3.1**	**4.5**	**21.2**
	Bourbon:												
14	First	22	.20	.46	22	65	1.8	6.4	8	10	3.0	3.6	27.0
15	Do	16	.20	.49	22	67	1.8	6.8	8	10	3.0	3.8	29.4
16	Do	13	.19	.49	22	67	1.6	6.2	7	9	3.0	3.9	27.8

[1] Calculated to volume of extract.

TABLE 1.—*Analyses of vanilla extracts (tincture of vanilla U. S. P.) made in the laboratory*—Continued.

Serial No.	Kind and quality of bean.	Length of bean.	Vanillin.	Normal lead number.	Color value.				Per cent of total color in lead filtrate.		Ratio of red to yellow.		Per cent of total color insoluble in amyl alcohol.
					Extract (total color).		Lead filtrate.						
					Red.	Yellow.	Red.	Yellow.	Red.	Yellow.	Extract.	Lead filtrate.	
		cm.	*Gms. per 100 cc.*						*Per cent.*	*Per cent.*			*Per cent.*
	Bourbon—Contd.												
17	Second	22	.15	.55	22	68	1.4	5.8	6	9	3.1	4.1	29.4
18	Do	17	.20	.62	55	127	2.4	6.8	4	5	2.3	2.8	24.4
19	Do	11	.21	.63	30	110	1.8	8.2	6	7	3.7	4.6	29.4
20	Third	22	.22	.52	25	80	2.0	7.0	8	9	3.2	3.5	27.8
21	Do	15	.20	.44	25	97	1.4	7.0	6	7	3.9	5.0	27.0
22	Do	10	.17	.55	30	104	2.0	7.6	7	7	3.5	3.8	26.3
23	Third (splits)	19	.19	.49	44	115	2.0	8.0	5	7	2.6	4.0	23.3
24	Fourth	22	.15	.48	30	90	1.8	6.2	6	7	3.0	3.4	26.5
25	Do	15	.14	.50	25	98	1.8	6.8	7	7	3.9	3.8	20.3
26	Do	10	.13	.54	25	90	2.0	6.8	8	7	3.6	3.4	27.0
27	Fifth	22	.14	.52	32	107	2.0	7.8	6	7	3.3	3.9	21.3
28	Do	15	.15	.56	40	115	2.0	6.8	5	6	2.9	3.4	21.7
29	Do	10	.16	.55	32	97	1.8	7.6	6	8	3.0	4.2	26.3
	Maximum	22	.22	.63	55	127	2.4	8.2	8	10	3.9	5.0	30.3
	Minimum	10	.13	.44	22	65	1.4	5.8	4	5	2.3	2.8	21.3
	Average	**16**	**.18**	**.52**	**30**	**94**	**1.9**	**7.0**	**6**	**8**	**3.2**	**3.8**	**26.6**
	Seychelles:												
30	First	20	.19	.46	22	80	1.0	5.0	4	6	3.6	5.0	23.3
31	Do	15	.19	.41	23	77	1.2	5.8	5	8	3.3	4.8	22.7
32	Do	10	.20	.52	22	77	1.4	6.0	6	8	3.5	4.3	27.0
33	Third	22	.21	.45	25	87	1.4	6.6	6	8	3.5	4.7	25.1
34	Do	15	.19	.51	47	117	2.2	8.8	5	8	2.5	4.0	25.1
35	Do	11	.19	.54	39	115	1.8	8.2	5	7	2.9	4.6	27.0
36	Fourth	22	.21	.51	50	162	3.4	14.6	7	9	3.2	4.3	29.4
37	Do	15	.18	.60	38	120	2.0	8.2	5	7	3.1	4.1	25.0
38	Do	10	.16	.59	35	125	1.8	8.2	5	7	3.6	4.6	25.6
	Maximum	22	.21	.60	50	162	3.4	14.6	7	9	3.6	5.0	29.4
	Minimum	10	.16	.45	22	77	1.0	5.0	4	6	2.5	4.0	22.7
	Average	**16**	**.19**	**.51**	**33**	**107**	**1.8**	**7.9**	**5**	**8**	**3.2**	**4.5**	**25.6**
	Madagascar:												
39	Second	23	.23	.42	25	87	1.6	7.2	6	8	3.5	4.5	24.3
40	Do	17	.23	.40	25	85	1.4	6.2	6	7	3.4	4.4	25.0
41	Do	11	.19	.46	31	85	1.8	7.6	6	9	2.7	4.2	23.2
42	Third	22	.21	.55	30	102	2.4	8.4	7	8	3.4	3.5	27.8
43	Do	15	.21	.61	47	147	1.8	9.2	4	6	3.1	5.1	27.8
44	Do	12	.16	.63	42	120	1.8	9.2	4	8	2.9	5.1	28.6
45	Fourth	20	.30	.49	42	148	2.6	11.5	6	8	3.5	4.4	30.3
46	Do	15	.24	.41	30	110	1.8	9.0	6	8	3.3	5.0	27.8
47	Do	12	.24	.51	37	115	2.4	10.0	7	9	3.1	4.2	26.3
	Maximum	23	.30	.63	47	148	2.6	11.5	7	9	3.5	5.1	30.3
	Minimum	11	.16	.40	25	85	1.4	6.2	4	6	2.7	3.5	23.2
	Average	**16**	**.22**	**.50**	**34**	**111**	**2.0**	**8.7**	**6**	**8**	**3.2**	**4.5**	**26.8**
	Comores:												
48	First[1]	21	.19	.52	30	90	1.4	6.6	5	7	3.0	4.7	26.3
49	Do	15	.18	.54	30	107	1.8	6.4	6	6	3.6	3.6	28.6
50	Do	11	.18	.59	25	95	2.0	7.0	8	8	3.8	3.5	25.0
51	Third[1]	19	.19	.59	35	115	2.2	7.6	6	7	3.3	3.5	25.6
52	Do	15	.17	.59	30	107	1.8	7.4	6	7	3.6	4.1	25.0
53	Do	10	.15	.68	30	92	2.2	7.4	7	8	3.1	3.4	28.6
54	Fourth[1]	19	.12	.74	40	140	2.4	9.2	6	7	3.5	3.4	26.3
55	Do	14	.12	.71	32	105	2.4	8.4	7	8	3.3	3.5	27.0
56	Do	10	.15	.64	37	105	2.4	8.6	6	8	2.8	3.6	25.6
57	First[2]	21	.31	.40	26	77	1.8	7.0	7	9	3.0	3.9	26.3
58	Do	15	.22	.52	27	87	1.4	7.4	5	8	3.2	5.3	28.6
59	Second[2]	15	.25	.48	40	134	2.6	12.6	7	9	3.3	4.8	35.7
60	Fourth[2]	15	.12	.73	37	107	2.0	9.6	7	9	2.9	4.8	30.3
61	First[3]	15	.20	.48	22	70	1.4	6.0	6	8	3.2	4.3	24.4
62	Third[3]	15	.20	.55	26	77	1.4	6.0	5	8	3.0	4.3	20.4
63	Fourth[3]	15	.16	.65	27	77	1.4	6.2	5	8	2.9	4.4	23.7
	Maximum	21	.31	.74	40	140	2.6	12.6	8	9	3.8	5.3	30.3
	Minimum	10	.12	.40	22	70	1.4	6.0	5	6	2.8	3.4	20.4
	Average	**15**	**.18**	**.59**	**31**	**99**	**1.9**	**7.7**	**6**	**8**	**3.2**	**4.1**	**26.7**

[1] Pomoni, Anjouan.
[2] Comoro.
[3] Mayotte.

TABLE 1.—*Analyses of vanilla extracts (tincture of vanilla U. S. P.) made in the laboratory*—Continued.

Serial No.	Kind and quality of bean.	Length of bean.	Vanillin.	Normal lead number.	Color value.				Per cent of total color in lead filtrate.		Ratio of red to yellow.		Per cent of total color insoluble in amyl alcohol.
					Extract (total color).		Lead filtrate.						
					Red.	Yellow.	Red.	Yellow.	Red.	Yellow.	Extract.	Lead filtrate.	
		cm.	*Gms. per 100 cc.*						*Per cent.*	*Per cent.*			*Per cent.*
	South American:												
64	First	....	.23	.50	50	155	2.6	10.4	5	6	3.1	4.0	29.4
65	Second	....	.19	.58	42	130	2.4	8.4	6	6	3.1	3.5	20.0
66	Splits	....	.22	.49	46	117	1.8	6.8	4	6	2.5	3.8	20.4
	Maximum	....	.23	.58	50	155	2.6	10.4	6	6	3.1	4.0	29.4
	Minimum	....	.19	.49	42	117	1.8	6.8	4	6	2.5	3.5	20.0
	Average	....	**.21**	**.52**	**46**	**134**	**2.3**	**8.5**	**5**	**6**	**2.9**	**3.8**	**23.3**
	Ceylon:												
67	First	20	.08	.67	40	145	1.4	6.4	4	4	3.6	4.6	22.7
68	Do	17	.08	.62	42	147	3.8	15.6	9	11	3.5	4.1	35.7
69	Do	12	.07	.57	61	195	7.6	32.6	12	17	3.2	4.3	50.0
	Maximum	20	.08	.67	61	195	7.6	32.6	12	17	3.6	4.6	50.0
	Minimum	12	.07	.57	40	145	1.4	6.4	4	4	3.2	4.1	22.7
	Average	**16**	**.08**	**.62**	**48**	**162**	**4.3**	**18.2**	**8**	**11**	**3.4**	**4.3**	**36.1**
	Java:												
70	Fourth	20	.23	.61	45	177	2.4	10.4	5	6	3.9	4.3	32.2
71	Do	14	.24	.44	44	142	3.2	13.4	7	9	3.2	4.2	35.7
72	Do	10	.22	.44	44	130	3.0	12.4	7	10	3.0	4.1	35.7
	Maximum	20	.24	.61	45	177	3.2	13.4	7	10	3.9	4.3	35.7
	Minimum	10	.22	.44	44	130	2.4	10.4	5	6	3.0	4.1	32.2
	Average	15	**.23**	**.50**	**44**	**150**	**2.9**	**12.1**	**6**	**8**	**3.4**	**4.2**	**34.5**
	Tahiti:												
73	——	....	.11	.50	17	50	.6	3.5	4	7	3.0	5.8	18.8
74	——	....	.11	.44	15	40	.6	3.1	4	8	2.7	5.2	16.0
	Average	....	**.11**	**.47**	**16**	**45**	**.6**	**3.3**	**4**	**8**	**2.9**	**5.5**	**17.4**
	Vanillons:												
75	——	....	.06	.52	42	107	1.4	6.6	3	6	2.5	4.7	22.2
	Tonka beans:												
	Angostura—												
76	Prime	....	(1)	.11	5	18	.5	2.4	10	13	3.6	4.8	30.3
77	Bloaters	....	(2)	.11	5	19	.5	2.4	10	13	3.8	4.8	31.2
	Average	....	(3)	**.11**	**5**	**19**	**.5**	**2.4**	**10**	**13**	**3.7**	**4.8**	**30.8**
	All analyses:[4]												
	Maximum	23	.31	.74	56	177	3.4	14.6	8	10	3.9	5.8	35.7
	Minimum	10	.11	.40	15	40	.6	3.1	4	5	2.3	2.8	16.0
	Average	**16**	**.19**	**.54**	**32**	**102**	**1.8**	**7.6**	**6**	**8**	**3.2**	**4.2**	**25.5**

[1] Coumarin, 0.27 per cent.

[2] Coumarin, 0.22 per cent.

[3] Coumarin, 0.25 per cent.

[4] Excluding Ceylon vanilla, Vanillons, and Tonka beans.

TABLE 2.—*Analyses of extracts made from residues from vanilla extracts (Table 1) by soaking in 60 per cent alcohol.*

[Results calculated to volume of U. S. P. extract.]

Serial number.	Kind and quality of bean.	Length of bean.	Vanillin.	Normal lead number.	Color value. Extract (total color).		Color value. Lead filtrate.		Per cent of total color in lead filtrate.		Ratio of red to yellow.	
					Red.	Yellow.	Red.	Yellow.	Red.	Yellow.	Extract.	Lead filtrate.
	Mexican:	*cm.*	*Grams per 100 cc.*						*Per cent.*	*Per cent.*		
1	First......	22	0.03	0.05	7	26	0.20	0.8	3	3	1:3.7	1:4.0
2	Do....	17	.03	.05	9	28	.30	1.0	3	3	3.1	3.3
3	Second....	22	.02	.11	16	50	.40	1.7	3	3	3.1	4.2
4	Do....	19	.04	.09	11	41	.25	1.3	2	3	3.7	5.2
5	Do....	15	.04	.06	10	32	.25	1.1	3	3	3.2	4.4
6	Third.....	23	.03	.05	8	28	.25	1.0	3	3	3.5	4.0
7	Do....	19	.02	.05	4	21	.25	1.0	6	5	5.2	4.0
8	Do....	15	.02	.06	5	23	.20	1.0	4	4	4.6	5.0
9	Third (splits)..	21	.03	.07	13	37	.35	1.3	3	4	2.8	3.7
10	Fourth....	20	.03	.06	6	24	.25	1.0	4	4	4.0	4.0
11	Fifth......	22	.03	.06	6	22	.20	1.0	3	5	3.7	5.0
12	Do....	16	.03	.07	7	23	.30	1.0	4	4	3.3	3.3
13	Cuts......	....	.02	.05	9	25	.25	1.1	3	4	2.8	4.4
	Bourbon:											
14	First......	22	.05	.05	10	30	.25	1.3	2	4	3.0	5.2
15	Do....	16	.05	.05	9	26	.35	1.1	4	4	2.9	3.1
16	Do....	13	.05	.06	8	24	.35	1.2	4	5	3.0	3.4
17	Second....	22	.03	.05	13	40	.30	1.1	2	3	3.1	3.7
18	Do....	17	.03	.05	13	33	.30	1.0	2	3	2.5	3.3
19	Do....	11	.04	.05	10	31	.30	1.1	3	4	3.1	3.7
20	Third.....	22	.05	.07	13	37	.40	1.8	3	5	2.8	4.5
21	Do....	15	.04	.04	9	31	.35	1.2	4	4	3.4	3.4
22	Do....	10	.03	.04	8	27	.25	1.0	3	4	3.4	4.0
23	Third (splits)..	19	.04	.05	9	31	.20	1.2	2	4	3.4	6.0
24	Fourth....	22	.04	.06	8	30	.25	1.3	3	4	3.7	5.2
25	Do....	15	.03	.04	6	25	.25	1.1	4	5	4.2	4.4
26	Do....	10	.03	.05	6	24	.25	1.0	4	4	4.0	4.0
27	Fifth......	22	.04	.08	13	47	.30	1.8	2	4	3.6	6.0
28	Do....	15	.04	.08	14	38	.30	1.5	2	4	2.7	5.0
29	Do....	10	.03	.03	11	30	.25	1.3	2	4	2.7	5.2
	Seychelles:											
30	First......	20	.04	.04	8	30	.20	1.0	3	3	3.7	5.0
31	Do....	15	.04	.04	8	36	.20	1.1	3	3	4.5	5.5
32	Do....	10	.03	.04	8	26	.15	.8	2	3	3.2	5.3
33	Third.....	22	.04	.04	8	28	.20	1.0	3	4	3.5	5.0
34	Do....	15	.04	.06	8	30	.20	1.3	3	4	3.7	6.5
35	Do....	11	....	....	....	....	....	....	....	....	....	....
36	Fourth....	22	.04	.05	11	34	.50	1.5	5	4	3.1	3.0
37	Do....	15	.04	.06	16	51	.40	1.7	3	3	3.2	4.2
38	Do....	10	.03	.04	9	30	.20	1.1	2	4	3.3	5.5
	Madagascar:											
39	Second....	23	.05	.04	13	48	.30	1.1	2	2	3.7	3.7
40	Do....	17	.05	.05	14	49	.30	1.1	2	2	3.5	3.7
41	Do....	11	.03	.05	9	30	.25	1.0	3	3	3.3	4.0
42	Third.....	22	.06	.06	13	47	.35	1.3	3	3	3.6	3.7
43	Do....	15	.03	.05	9	26	.25	1.3	3	5	2.9	5.2
44	Do....	12	.04	.08	11	36	.35	1.3	3	4	3.3	3.7
45	Fourth....	20	.04	.04	10	26	.30	1.4	3	5	2.6	4.7
46	Do....	15	.04	.06	9	30	.30	1.3	3	4	3.3	4.3
47	Do....	12	.03	.05	13	34	.40	1.7	3	5	2.6	4.2
	Comores:											
48	First [1].....	21	.03	.04	8	27	.25	1.1	3	4	3.4	4.4
49	Do....	15	.02	.04	8	28	.20	1.1	2	4	3.5	5.5
50	Do....	11	.02	.04	8	25	.25	.8	3	3	3.1	3.2
51	Third [1]....	19	.03	.04	8	26	.20	1.3	3	5	3.2	6.5
52	Do....	15	.03	.05	7	25	.20	1.1	3	4	3.6	5.5
53	Do....	10	.01	.04	6	21	.20	1.0	3	5	3.5	5.0
54	Fourth [1]..	19	.04	.07	10	36	.35	1.6	4	4	3.6	4.6
55	Do....	14	.03	.06	9	34	.30	1.5	3	4	3.8	5.0
56	Do....	10	.03	.05	8	30	.25	1.3	3	4	3.7	5.2
57	First [2].....	21	.04	.04	11	42	.20	1.3	2	3	3.8	6.5
58	Do....	15	.02	.03	7	24	.20	1.0	3	4	3.4	5.0
59	Second [2]...	15	.07	.06	17	62	.35	2.2	2	4	3.6	6.3
60	Fourth [2]..	15	.03	.05	7	27	.30	1.1	4	4	3.9	3.7

[1] Pomoni, Anjouan.

[2] Comoro.

Table 2.—*Analyses of extracts made from residues from vanilla extracts (Table 1) by soaking in 60 per cent alcohol*—Continued.

Serial number.	Kind and quality of bean.	Length of bean.	Vanillin.	Normal lead number.	Color value.				Per cent of total color in lead filtrate.		Ratio of red to yellow.	
					Extract (total color).		Lead filtrate.				Extract.	Lead filtrate.
					Red.	Yellow.	Red.	Yellow.	Red.	Yellow.		
	Comores—Contd.	*cm.*	*Grams per 100 cc.*						*Per cent.*	*Per cent.*		
61	First [1]	15	.04	.07	13	41	.25	1.6	2	4	3.2	6.4
62	Third [1]	15	.04	.09	15	46	.25	1.6	2	3	3.1	6.4
63	Fourth [1]	15	.03	.07	10	33	.20	1.3	2	4	3.3	6.5
	South American:											
64	First		.04	.04	11	40	.30	1.5	3	4	3.6	5.0
65	Second		.04	.07	13	48	.25	1.6	2	3	3.7	6.4
66	Splits		.04	.05	11	40	.25	1.2	2	3	3.6	4.8
	Ceylon:											
67	First	20	.03	.07	6	24	.20	1.1	3	5	4.0	5.5
68	Do	17	.02	.06	8	30	.30	1.7	4	6	3.7	5.7
69	Do	12	.01	.04	6	25	.25	1.6	4	6	4.2	6.4
	Java:											
70	Fourth	20	.05	.04	7	29	.45	1.6	6	6	4.1	3.6
71	Do	14	.04	.04	7	28	.25	1.4	4	5	4.0	5.6
72	Do	10	.03	.04	4	23	.28	1.3	7	6	5.7	4.6
	Tahiti:											
73	——		.02	.05	7	23	.25	.9	4	4	3.3	3.6
74	——		.01	.05	8	29	.20	.8	3	3	3.6	4.0
	Vanillons:											
75	——		.01	.06	15	46	.25	1.2	2	3	3.1	4.8
	Tonka beans:											
76	Angostura prime		.00	.04	1	6	.10	.5	10	8	6.0	5.0
77	Angostura bloaters		.00	.04	1	6	.10	.4	10	7	6.0	4.0
	All analyses:											
	Maximum		.07	.11	17	62	.50	2.2	7	6	5.7	6.5
	Minimum		.01	.03	4	21	.15	.8	2	2	2.5	3.0
	Average		.03	.05	9	32	.27	1 2	3	4	3.4	4.6

[1] Mayotte.

DISCUSSION OF RESULTS.

The extracts from the three lengths of Ceylon beans were so abnormal and variable in composition as to indicate that either the beans had been cured by an unusual process or else exhausted and treated in some manner to disguise the fact. The vanillin in all of the samples was abnormally low, while the percentage of color left in the lead filtrate, while abnormally low in the extract from the long beans, was abnormally high in that from the short beans. This bean is uncommon in our market.

Vanillin.—The range in vanillin content (0.11–0.31 gram per 100 cc) is somewhat greater than has usually been thought possible. Leach states that an extract containing as high as 0.25 per cent of vanillin is suspicious, which is doubtless true of extracts made on a large scale, just as abnormal percentages of fat which may occur in the milk of individual cows will not be found in the milk of a mixed herd. The extract from the residue corresponding to the sample containing the maximum percentage of vanillin (0.31) gave 0.04 additional, making the total 0.35.

The minimum figure (0.11) was found in the Tahiti extracts, which were prepared from undried beans. Had dried beans been used, as is the custom in the trade, the percentages of all the constituents would have been materially higher.

Normal lead number.—The variation in lead number (from 0.40–0.74) is less than that of any other of the constants. The constituents which give the precipitate appear to be more easily soluble than the vanillin and color, the second extraction yielding an average normal lead number of only 0.05. This determination not only serves to distinguish a true extract from a solution of vanillin, but also is of value in conjunction with the determination of coumarin as a means of detecting the presence of Tonka extract.

Color values of extract and lead filtrate.—The color appears to be the most variable and most difficultly extractable constituent. As a means of detecting caramel, the per cent of color left in the lead filtrate is most significant. The maximum found in these samples (excluding Ceylon) was 8 per cent red and 10 per cent yellow, whereas one of the samples colored with caramel sent out during the year 1911 by Mr. Hiltner, as associate referee on extracts, gave, respectively, 20 per cent and 37 per cent, and commercial extracts have given similar figures. The ratio of red to yellow in the extract and lead filtrate is also of value, as caramel solution shows a lower ratio, or, in other words, is redder. The minimum ratio found in our extracts was 1:2.3, as compared with 1:1.9 in Mr. Hiltner's sample.

Per cent of color insoluble in amyl alcohol.—The results corroborate those obtained in the determination of the per cent of color in the lead filtrate, with the advantage that they are not so cumbersome and do not require a tintometer. On the other hand, this determination requires an additional process, whereas the color values of the lead filtrate are obtained incidentally in the determination of vanillin and lead number.

Influence of grade of bean on the constants of the extract.—In the following table are given the average figures for extracts from the five grades of all the varieties of vanilla beans, excluding South America, Java, and Tahiti, of which there were only one or two grades, and Ceylon, which was abnormal:

TABLE 3.—*Averages of analyses of U. S. P. vanilla extracts according to grade of bean.*

Grade of bean.	Number of samples.	Vanillin.	Normal lead number.	Color value.				Per cent of total color in lead filtrate.		Ratio of red to yellow.		Per cent of total color insoluble in amyl alcohol.
				Extract (total color).		Lead filtrate.				Extract.	Lead filtrate.	
				Red.	Yellow.	Red.	Yellow.	Red.	Yellow.			
		Grams per 100 cc.						*Per cent.*	*Per cent.*			*Per cent.*
First	14	0.20	0.49	24	77	1.5	6.1	6	8	1:3.2	1:4.2	25.7
Second	10	.20	.53	31	92	1.7	7.2	6	8	3.1	4.4	25.3
Third	16	.19	.55	32	104	1.8	7.6	6	7	3.3	4.3	25.7
Fourth	15	.17	.58	35	113	2.1	8.7	6	8	3.2	4.1	26.8
Fifth	5	.15	.59	35	111	1.9	7.5	5	7	3.1	4.0	22.5

These figures show, from the lowest to the highest grade, a marked increase of vanillin and a marked decrease of normal lead number and color value. In this connection it should be noted that the Tahiti extracts, the cheapest of all, while containing the lowest amounts of vanillin, are exceptional in that the color values are also lowest, while the normal lead numbers are intermediate. It must, therefore, be understood that the conclusions reached are relative, applying only to the different grades of the same or related varieties.

Influence of length of bean on the constants of the extract.—The average results of analyses of extracts from long, medium, and short beans, excluding South American and Tahiti, which were not sorted by lengths, and Ceylon, which were abnormal, are shown in the following table:

TABLE 4.—*Averages of analyses of U. S. P. vanilla extracts according to length of bean.*

Length of bean.	Number of samples.	Vanillin.	Normal lead number.	Color value.				Per cent of total color in lead filtrate.		Ratio of red to yellow.		Per cent of total color insoluble in amyl alcohol.
				Extract (total color).		Lead filtrate.				Extract.	Lead filtrate.	
				Red.	Yellow.	Red.	Yellow.	Red.	Yellow.			
cm.		*Grams per 100 cc.*						*Per cent.*	*Per cent.*			*Per cent.*
20-23	19	0.20	0.52	30	99	1.8	7.6	6	8	1 : 3.3	1 : 4.3	25.3
15-19	27	.18	.55	32	100	1.7	7.2	6	7	3.2	4.3	25.4
10-14	17	.18	.56	33	104	2.1	8.4	6	8	3.2	4.1	27.8

A slight increase in vanillin and decrease in normal lead number and color values from the shortest to the longest beans is noticeable. These differences, although not marked, point to a slight inferiority of the shorter beans.

VANILLA EXTRACTS PREPARED WITH DIFFERENT MENSTRUA IN THE LABORATORY.

PREPARATION OF EXTRACTS.

Six extracts were made from each of three typical varieties of beans, viz, Mexican, Bourbon, and Tahiti, using the following menstrua: (1) Sixty per cent alcohol only; (2) 60 per cent alcohol and sugar (U. S. P.); (3) 60 per cent alcohol and glycerin; (4) 35 per cent alcohol only; (5) 35 per cent alcohol and sugar; (6) 35 per cent alcohol and glycerin. In all cases two liters of extract were made from 200 grams of the ground material.

The extracts made with 60 per cent alcohol and sugar were the usual U. S. P. preparations, as were also those made with 35 per cent alcohol and sugar, except as regards the strength of the alcohol.

In preparing the glycerin extracts 200 cc of glycerin were first mixed with the ground beans, after which the manipulation was the same as in the U. S. P. process, except that the material, after maceration and draining off the liquid, was pounded in a mortar with 200 grams of sand instead of sugar. One set of these glycerin extracts was made with 60 per cent alcohol, the other with 35 per cent alcohol.

The alcohol extracts were made with 60 per cent and 35 per cent alcohol, without the addition of either sugar or glycerin, the manipulation in these also being the same as in the U. S. P. process, except for the substitution of 200 grams of sand for sugar.

The preparation of the extracts with 35 per cent alcohol, both with and without sugar or glycerin, was highly unsatisfactory, owing to the extraction of gelatinous material, which clogged the percolators. It was found necessary to loosen up the material repeatedly, and as a consequence the percolate was a muddy liquid containing seeds and other matters in suspension. These mechanical impurities were partially removed by long settling and filtration of the supernatant liquid. This process could not be carried out to advantage on a commercial scale, and consequently the extracts do not represent products on the market, but rather abnormal preparations useful in illustrating the effects of diluting the alcohol beyond a certain limit.

In all cases second extracts were prepared from the residues from the first extracts by soaking in 60 per cent alcohol, as already described.

ANALYSES OF THE EXTRACTS.

The analyses of the first extracts appear in Table 5, and of the extracts made from the residues in Table 6.

TABLE 5.—*Analyses of vanilla extracts prepared with different menstrua in the laboratory.*

Kind of bean and menstruum.	Vanillin.	Lead number.	Color value.				Per cent of total color in lead filtrate.		Ratio of red to yellow.		Total color insoluble in amyl alcohol
			Extract (total color).		Lead filtrate.[1]				Extract.	Lead filtrate.	
			Red.	Yellow.	Red.	Yellow.	Red.	Yellow.			
	Gms. per 100 cc.						*Per cent.*	*Per cent.*			*Per cent.*
Mexican:											
60 per cent alcohol	0.19	0.70	33	108	1.8	7.1	5.5	6.9	1:3.1	1:3.9	18.6
60 per cent alcohol and sugar (U. S. P.)	.19	.65	35	108	2.0	8.6	5.7	8.4	2.9	4.3	24.4
60 per cent alcohol and glycerin	.16	.69	41	123	2.0	8.8	4.9	7.2	3.0	4.4	22.7
35 per cent alcohol	.17	.82	31	75	1.8	6.6	5.8	8.8	2.4	3.7	27.8
35 per cent alcohol and sugar	.17	.79	28	75	2.0	7.5	7.1	10.0	2.4	3.8	38.5
35 per cent alcohol and glycerin	.18	.80	40	90	2.0	7.5	5.0	8.3	2.3	3.8	35.7
Bourbon:											
60 per cent alcohol	.19	.61	34	108	1.9	8.2	5.6	7.6	3.2	4.3	20.5
60 per cent alcohol and sugar (U. S. P.)	.19	.57	39	119	2.4	10.0	6.2	8.4	3.1	4.2	26.1
60 per cent alcohol and glycerin	.20	.58	43	133	2.0	9.6	4.7	7.2	3.1	4.8	25.0
35 per cent alcohol	.17	.71	30	73	1.8	7.0	6.0	9.6	2.4	3.9	34.5
35 per cent alcohol and sugar	.17	.66	31	78	2.2	8.0	7.1	10.3	2.4	3.6	45.4
35 per cent alcohol and glycerin	.19	.68	50	110	2.4	8.4	4.8	7.6	2.2	3.5	38.5
Tahiti:											
60 per cent alcohol	.11	.43	13	40	.6	2.6	4.6	6.5	3.1	4.3	14.3
60 per cent alcohol and sugar (U. S. P.)	.11	.44	15	40	.6	3.1	4.0	7.7	2.7	5.2	16.0
60 per cent alcohol and glycerin	.11	.43	24	53	.7	3.3	2.9	6.2	2.2	4.7	15.9
35 per cent alcohol	.09	.39	15	30	1.2	4.0	8.0	13.3	2.3	3.3	23.8
35 per cent alcohol and sugar	.09	.44	15	33	1.3	4.4	8.7	13.9	2.2	3.4	28.6
35 per cent alcohol and glycerin	.10	.40	15	33	.9	3.3	6.0	10.0	2.0	3.6	24.4

[1] Calculated to volume of extract.

TABLE 6.—*Analyses of extracts prepared from residues from vanilla extracts (Table 5) by soaking in 60 per cent alcohol.*

[Results calculated to volume of U. S. P. extract.]

Kind of bean and menstruum.	Vanillin.	Normal lead number.	Color value.				Per cent of total color in lead filtrate.		Ratio of red to yellow.	
			Extract (total color).		Lead filtrate.				Ex-tract.	Lead filtrate.
			Red.	Yellow.	Red.	Yellow.	Red.	Yellow.		
Mexican:	*Grams per 100 cc.*									
60 per cent alcohol	0.04	0.07	11	31	0.3	1.1	2.7	3.5	1:2.8	1:3.7
60 per cent alcohol and sugar	.04	.09	13	38	.3	1.2	2.3	3.2	2.9	4.0
60 per cent alcohol and glycerin	.04	.06	6	25	.3	1.05	5.0	4.2	4.2	3.5
35 per cent alcohol	.03	.05	......	......	......	......	......	......	......	......
35 per cent alcohol and sugar	.03	.05	......	......	......	......	......	......	......	......
35 per cent alcohol and glycerin	.03	.04	......	......	......	......	......	......	......	......
Bourbon:										
60 per cent alcohol	.04	.06	11	31	.3	1.2	2.7	3.9	2.8	4.0
60 per cent alcohol and sugar	.04	.06	11	30	.35	1.2	3.2	4.0	2.7	3.4
60 per cent alcohol and glycerin	.04	.04	8	24	.35	1.25	4.4	5.2	3.0	3.6
35 per cent alcohol	.03	.05	......	......	......	......	......	......	......	......
35 per cent alcohol and sugar	.05	.06	......	......	......	......	......	......	......	......
35 per cent alcohol and glycerin	.04	.04	......	......	......	......	......	......	......	......
Tahiti:										
60 per cent alcohol	.01	.03	6	19	.2	.8	3.3	4.2	3.2	4.0
60 per cent alcohol and sugar	.01	.05	8	29	.2	.8	2.5	2.8	3.6	4.0
60 per cent alcohol and glycerin	.01	.05	4	13	.2	.85	5.0	6.5	3.2	4.2
35 per cent alcohol	.01	.03	......	......	......	......	......	......	......	......
35 per cent alcohol and sugar	.01	.03	......	......	......	......	......	......	......	......
35 per cent alcohol and glycerin	.01	.03	......	......	......	......	......	......	......	......

INFLUENCE OF GLYCERIN AND SUGAR AND STRENGTH OF ALCOHOL ON COMPOSITION.

When the same strength of alcohol was employed the only noteworthy difference brought out by analysis was that the glycerin extracts were more strongly colored than when alcohol alone or alcohol and sugar were used. This explains in part why glycerin is so much used in the manufacture of commercial extracts. The percentages of vanillin and normal lead number are practically the same whether or not glycerin or sugar was used. The color values of the second extracts made from the residues from the glycerin extracts were lower than those of the others, corresponding to the excess of color in the first extracts.

The difference in the extractive power of 60 per cent and 35 per cent alcohol, while not noticeable as regards vanillin, is marked as regards the other constituents. The weaker alcohol gave higher normal lead numbers, lower color values of the extract and higher percentages of color remaining both in the lead filtrate and insoluble in amyl alcohol.

STANDARD VANILLA EXTRACT.

The following tentative limits of composition for standard vanilla extract (10 grams of beans to 100 cc) appear to be warranted by the results obtained:

Vanillin, 0.10 to 0.35 gram per 100 cc.

Normal lead number, 0.40 to 0.80.

Per cent of total color in lead filtrate, not more than 10 per cent red or 12 per cent yellow.

Ratio of red to yellow in the extract, not less than 1 : 2.2.

Color insoluble in amyl alcohol, not more than 40 per cent.

REPORT ON COCOA AND COCOA PRODUCTS.

By W. L. Dubois, *Associate Referee.*

The work on cocoa products this year was confined to further testing of the short method for the determination of ether extract which was presented at the 1910 meeting of the association, and to the determination of milk solids in milk chocolate.

Ether Extract by the Proposed Short Method.

A sample of cocoa was sent to the collaborators with the following memorandum:

INSTRUCTIONS FOR COOPERATIVE WORK.

The method for the rapid determination of fat in cocoa products was presented to the association at its 1910 meeting (see Bul. 137, p. 103, for statement of method).

Two objections were brought against this method by the collaborators. One was that the filtering offered opportunity for the evaporation of the solvent and tended to give high results. It was contended that if the product were centrifuged long enough and care exercised in pouring the solvent from the cocoa material packed in the bottle, no necessity for the use of filtering paper would arise, it being sufficient to merely pour the ether through a small funnel into the burette. The experience of the referee differed on this point, some samples of cocoa seeming to pack well and others appearing to have so much fine material that it was almost impossible to pour off the ether without contaminating the same with some cocoa, which, of course, would tend to high results. It is therefore desired that the collaborators this year test this point and report their opinion as to whether filtering is necessary or desirable.

The second point is regarding the effect of hot weather upon the determination, two laboratories reporting that on very hot days there was an apparent evaporation of ether. The referee has not observed that higher results were obtained on warm days than on cool days, but such may be the case in laboratories where the temperature becomes quite high. It is therefore desired that the collaborators test this point also, determining the fat in the sample submitted under as ideal conditions as regards temperature as possible, and also on several very warm days, noting the temperature of the laboratory each time and reporting results obtained, together with a statement of opinion as to the point under consideration.

ANALYTICAL RESULTS.

A table is presented herewith to show results obtained by the different workers on this sample:

Cooperative results on ether extract by the short method testing effect of temperature and necessity of filtration.

Analyst.	Filtered.	Not filtered.	By continuous extraction.		Temperature.	Remarks.
			Petroleum ether.	Ether.		
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	°*C.*	
C. I. Lott	23.09	22.80	....	....	....	
M. C. Albrech	....	23.01 23.04	....	....	29	
L. C. Mitchell	23.13 23.05	22.81 22.58	....	23.35 23.26	....	
N. Hendrickson	23.56 23.53	....	....	....	27	

Cooperative results on either extract by the short method testing effect of temperature and necessity of filtration—Continued.

Analyst.	Filtered.	Not filtered.	By continuous extraction.		Temperature.	Remarks.
			Petroleum ether.	Ether.		
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	°*C.*	
H. S. Bailey	24.45				29.5	
	23.43				29.5	
	23.43				22	
	23.86				24	
	23.06				24	
	25.07				29	
	24.63				29	
	23.85				20	
	23.20					
R. S. Hiltner		22.97			25	
	23.41				28	
		23.06			28	
				22.98		Knorr apparatus; sample not reextracted, fat not perfectly clear.
				22.87		Johnson extractor 4 hours, fat not perfectly clear.
				22.64		Leach-Hiltner method.
W. L. Dubois		22.64			18.5	
	24.49					
	24.39				29	
				23.37		Alundum thimble in Knorr apparatus.
			22.35			Do.

COMMENTS BY THE COLLABORATORS.

C. I. Lott: It seems to me important to stipulate that the ether used in this determination shall be at room temperature. If measured from a supply kept in the refrigerator, by the time the ether assumes the temperature of the room it will have expanded considerably so as to introduce a possible error from this source.

L. C. Mitchell: I see no use in filtering, as the sample was packed firmly.

M. C. Albrech: I have never found it necessary to filter, as I could always get the samples securely packed in the bottom of the bottle, so that the fat was entirely clear or was only slightly contaminated with cocoa material. In a large number of determinations I have frequently had one sample slightly contaminated with cocoa material and its duplicate entirely free, and there would be practically no difference in the duplicate results. I am strongly opposed to filtering the ether solution, as I think there is chance for error and evaporation, which errors are eliminated by avoiding filtration.

H. S. Bailey: I find that in ordinary summer temperature (85° to 90° F.) it is impossible to draw 50 cc of ether from a burette into an oil bottle and return the same to the burette without losing from 0.5 to 0.75 cc of the solvent, and when instead of an empty funnel a 7 cm filter paper was added the loss was three or four times as great. It appears to me, therefore, that it is impossible to filter the ether without introducing a very appreciable error in the determination. I think the fundamental ideas in the method are all right, and I see no reason why it should not be adopted for rapid work, if instead of filtering from the bottle into the burette this transfer be made as quickly as possible, and then, after noting the quantity of ether returned to the burette, the solvent with its fat will all be filtered into the tared flask.

R. S. Hiltner: It is evident that this method may be relied upon if the ether solution is poured directly into the burette and not filtered, and if the operation is conducted at a temperature not exceeding 25° C. and away from strong drafts. If the extracts are filtered the results will be erroneously high. Personally, I should prefer using a Johnson and Knorr extractor and extracting the sample for four hours or longer, because less personal attention is required and because the method is less sensitive to atmospheric and temperature conditions.

DISCUSSION.

From the results obtained by the collaborators it is apparent that filtering of the fat solution is inadvisable, and that when this feature is omitted sufficiently accurate results can be obtained to make the proposition applicable to cocoa when a determination is desired in a short period of time. The manipulation is doubtless equal to that involved in the continuous extraction method, but the advantage of being able to obtain a result in shorter time remains.

While this method seems to be satisfactory for cocoa, some results have been obtained in this laboratory during the past year which throw some doubt upon its applicability to milk chocolates. By referring to the table of analyses of milk chocolates given on page 163 it will be seen that the results on ether extract are considerably lower by the proposed method than those obtained by continuous extraction with ether in Sohxlet's apparatus for 18 hours. This is probably due to the inability of ether to dissolve all of the butter fat, which is doubtless surrounded, at least in part, by an envelope of proteid material. An experiment on milk powder alone by this method gave results which were extremely low. While quite a number of figures have been reported in the past to support this short method as a reliable procedure for the determination of fat in milk chocolate, it seems that it may not always be above criticism when applied to this class of products. Some milk chocolates will doubtless yield their total fat more easily than others, depending upon the method of manufacture. Those chocolates which are made by mixing liquid milk with chocolate liquor and evaporating the whole together will doubtless present the butter fat in a more finely divided condition and therefore more easily extracted by the simple shaking with ether.

In view of the several uncertainties and sources of error, it seems to the referee that the method in question can be recommended as only an approximate one, to be used in cases where a quick determination is desired. It would not be his opinion, therefore, that the method should be recommended to the association for provisional adoption at this time.

SUBSTITUTION OF PETROLEUM ETHER FOR SULPHURIC ETHER.

Regarding the matter of fat determination in general in cocoa products, it has seemed to the referee for some time that the substitution of petroleum ether for sulphuric ether would be advisable. A small amount of the extract obtained by sulphuric ether seems to be other than cocoa fat. In a number of samples of cocoa which were extracted in the referee's laboratory by both petroleum ether and sulphuric ether, a slightly higher result was obtained by the latter. The extracted material, however, did not completely redissolve in ether, a white residue being apparent which was soluble in hot alcohol. It was thought that this might be theobromin, which is somewhat soluble in sulphuric ether, but the quantity obtainable was so small that attempts to crystallize the substance were unsuccessful. Theobromin is insoluble in petroleum ether, and the extracts obtained with petroleum ether on the above samples were completely soluble in ether, containing none of this white material, the extract being apparently all cocoa butter. Since cocoa butter is perfectly soluble in petroleum ether, there would seem to be no objection to the substitution referred to, while it appears that more accurate results are obtained thereby. Results on five cocoas follow:

Comparison of ether extract results using sulphuric and petroleum ether.

Sulphuric ether.	Petroleum ether.
Per cent.	*Per cent.*
23.07	22.74
29.39	28.86
28.12	27.52
24.99	24.40
23.15	22.56

With petroleum ether no more fat was obtained by extracting the collaborative sample for twelve hours and then grinding and extracting an additional four hours, than by simply extracting four hours without regrinding. While this was the case with this particular cocoa sample, however, it might not follow that such a short process would completely remove the fat from such products as chocolate and milk chocolate, in which cases the regrinding would probably be necessary. The wisdom of substituting petroleum ether for sulphuric ether, however, would hold with such products as well as with cocoa. The referee would recommend that this substitution be made.

MILK SOLIDS IN MILK CHOCOLATE.

Attempt was made to estimate the milk solids in milk chocolate by determining lactose, butter fat, and casein. Lactose was determined by the method reported last year (Bul. 137, p. 101) and recommended for provisional adoption; butter fat was estimated by determining the Reichert-Meissl number on ether extract and assuming 24 as the number of butter in making the calculation. It is necessary to make some deduction for the Reichert-Meissl number of cocoa butter, which, although small, is still appreciable. This number was determined on several cocoa butters obtained from manufacturers of cocoa products, and the results are given in the table. It was first thought that possibly the Koettstorfer number would assist in estimating butter fat, but it was found that the small amount of butter fat present would not introduce sufficient variation in this number to make it of value in the calculation. The results obtained on commercial cocoa butters, however, are included in the table:

Results on commercial cocoa butters.

No. of sample.	Reichert-Meissl number.	Koettstorfer number.
1	0.30	195.2
2	.20	193.3
3	.29	192.8
4	.60	193.6
5	.20	194.4
6	.10	192.4
7	.22	193.5
8	.10	194.8
9	.12	193.8
10	.60	193.0
11	.61	193.5

The average of these 11 Reichert-Meissl numbers is 0.30, but for the calculations on chocolate samples the value 0.50 was taken as probably representing a good average of commercial cocoa butter.

Casein was determined by extracting the fat from 3 grams of sample by shaking with ether, centrifuging, and drawing off the solvent. The residue was then treated three times with 30 cc of 1 per cent solution of sodium phosphate (Na_3PO_4), the bottle being heated to 60° C. each time for half an hour and shaken from time to time during this interval. It was then centrifuged and the supernatant liquid poured into a 200 cc flask. After the three extractions the volume was made up to 200 cc and 50 cc removed to a beaker to which 50 cc of water and 1 cc of 50 per cent solution of trichloracetic acid were added, the solution brought to 100° C., and the precipitate received on a tared filter, washed with hot water, dried, and weighed, and the casein computed from the increase in weight. This is doubtless a crude method for making this determination, since it was impossible to obtain a precipitate entirely free from cocoa tissue. The results proved approximate, however.

A sample of milk chocolate was prepared in the laboratory after the following formula:

	Per cent.
Chocolate	28
Sucrose	36
Milk powder	24
Cocoa butter	12

Besides this sample, three lots of milk chocolate were obtained from as many manufacturers, who stated the percentage of milk solids therein. Analyses of laboratory and commercial samples appear in the table:

Analyses of commercial and laboratory samples of milk chocolate.

Kind of chocolate.	Milk solids.	Lactose.	Fat.		Reichert-Meissl number (fat).	Koettstorfer number (fat).	Butter fat.	Casein.	Milk solids.
			Sohx.	Short.					
	Per ct.	*Per ct.*	*Per ct.*	*Per ct.*			*Per ct.*	*Per ct.*	*Per ct.*
Laboratory	24	10.6	33.66	30.16	5.5	200	7.0	8.8	26.4
Huyler's	10	3.4	33.02	30.7	2.9	196.4	3.3	2.30	9.1
Peter's	17.4	6.07	33.25	31.0	4.5	199.3	5.5	7.1	18.66
Penna	17.6	6.20	32.42	30.5	4.0	200	4.7	7.2	18.1

It would seem from these results that the milk solids calculated from the determinations made are too high in every case but one. This may be due to contamination of the casein, as pointed out before, but there was not time for the referee to carry this investigation further.

REPORT ON TEA AND COFFEE.

By M. E. Jaffa, *Associate Referee.*

In accordance with the recommendations of last year the cooperative work during the current year on methods of analysis of tea and coffee has been confined mainly to the determinations of thein and caffein. The following chemists collaborated in the work: J. A. Cummings and F. C. Woodruff, New York, N. Y.; A. L. Knisely, Portland, Oreg.; and G. R. Stewart and A. R. Mehrtens, Berkeley, Cal.

The methods employed in making these investigations are as follows:

METHODS OF ANALYSES.

TEA.

Determination of insoluble leaf and extract.—Method of Doolittle and Woodruff, Proceedings of 1906, Bulletin 105, page 48.

Determination of thein.—

(1) Dvorkovitsch method, Bulletin 107, Revised, page 150; (2) Doolittle and Woodruff method, Proceedings of 1906, Bulletin 105, page 50; (3) Modification of Stahlschmidt's method: Boil 6 grams of finely powdered tea in a flask with several successive portions of water for 10 minutes each, and make up the combined aqueous extracts thus obtained to 600 cc with water. Add 4 grams of powdered lead acetate to the decoction, then boil for 10 minutes, using a reflux condenser or making up the loss by occasional addition of water. Then pour the solution upon a dry filter and evaporate 500 cc of the filtrate, corresponding to 5 grams of the tea, to about 50 cc and add enough sodium phosphate to precipitate the remaining lead. Filter the solution, and thoroughly wash the precipitate, the filtrate and washings being evaporated to about 40 cc. Finally, extract the solution thus concentrated with chloroform in a separatory funnel at least four times and evaporate the chloroform extract to dryness, leaving the caffein, which is dried to constant weight at 75° and weighed.

Determination of caffein.—

(1) The Gorter method (Proceedings in 1910, Bul. 137, p. 106); (2) method of Lendrich and Nottbohm (Zts. Nahr. Genussm., 1909, 17. 241; Proceedings of 1910, Bul. 137, p. 107). The following additional details were given in the direction for this year's work for the final steps of the method: "Evaporate the filtrate and washings to about 50 cc, make alkaline with sodium hydroxid, and extract four times with 25 cc, 20, 15, and 15 cc of chloroform. Evaporate in tared dish, dry for 30 minutes at 100 C. and weigh as caffein. Transfer the caffein to a Kjeldahl flask with a small amount of hot water and determine nitrogen in the residue as a check on its purity. (N×3.464 equals caffein.)"

ANALYTICAL RESULTS.

Cooperative work on caffein in coffee.

Collaborators.	Sample.	Gorter method.		Lendrich and Nottbohm method.	
		Gravimetric.	N×3.464.	Gravimetric.	N×3.464.
		Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
J. A. Cummings, New York, N. Y.	1. Decaffinated	0.43	....	0.13	0.09
		.40	0.15	.15	....
		.35	.10	....	....
	2. Costa Rica	1.59	1.25	.56	.57
		1.57	1.23	.77	.76
		1.78	1.26	....	....
	3. Java	1.50	....	.96	.94
		1.42	1.16	1.05	1.03
		1.44	1.18	....	....
	4. Mocha	1.48	1.12	.84	.80
		1.52	1.22	1.10	1.10
		1.49	1.19	....	....
G. R. Stewart and A. R. Mehrtens, Berkeley, Cal.	1. Decaffinated	.67	.15	.11	.09
		.48	.15	.12	.08
	2. Costa Rica	1.57	1.22	.62	.57
		1.64	1.26	.71	.65
	3. Java	1.37	1.13	.78	.74
		1.35	1.12	.62	.59
	4. Mocha	1.40	1.09	.54	.51
		1.46	1.15	.55	.53
					1.12
	5. Salvador	1.50	1.24	.86	.80
		1.57	1.29	.80	.80

Cooperative work on tea.

Collaborators.	Sample.	Doolittle and Woodruff method.			Dvorkovitsch method.		Modified Stahlschmidt method.	
		Insoluble leaf.	Caffein.		Caffein.		Caffein.	
			Gravimetric.	N× 3.464.	Gravimetric.	N× 3.464.	Gravimetric.	N× 3.464.
		Per ct.	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*
F. O. Woodruff, New York, N. Y.	6. English Breakfast.	44.9	2.77	....	....	....	3.38	....
		45.1	2.93	....	2.90	....	3.22	....
	7. Oolong...........	46.2	3.29	....	....	....	3.73	....
		46.8	3.05	....	3.19	....	3.63	....
		....	3.15	....	....	....	....	....
A. L. Knisely, Portland, Oreg.	6. English Breakfast.	54.42	2.06	....	1.95	....	2.94	2.70
		54.75	1.74	....	Lost.	....	Lost.	....
	7. Oolong...........	48.76	1.92	....	1.91	....	3.16	2.93
		48.58	1.93	....	1.74	....	3.17	2.95
G. R. Stewart and A. R. Mehrtens, Berkeley, Cal.	6. English Breakfast.	52.35	2.90	2.31	2.74	2.56	3.01	2.84
		52.30	2.92	2.44	2.63	2.52	3.00	2.88
	7. Oolong...........	47.24	2.99	2.90	3.30	3.06	3.36	3.24
		47.32	3.28	3.21	3.31	3.16	3.35	3.24
		....	3.22	3.16	....	....	....	....

COMMENTS OF COLLABORATORS.

ON COFFEE.

J. A. Cummings: Commenting on the Gorter and the Lendrich and Nottbohm methods for the determination of caffein in coffee I would say that the Gorter method is the shorter, but it yields impure caffein, due to some colored substance which remains in solution after the cotton plug filtration. It was difficult in carrying out the Lendrich and Nottbohm method to get rid of the manganese dioxid by filtration, as some of it washed through the paper. It seems, too, that the carbon tetrachlorid extraction in this method yields a smaller percentage of caffein than does the chloroform extraction in the Gorter method. One explanation may be that the time for extraction in the Lendrich and Nottbohm method is not long enough for the complete extraction of the large amount of coffee used.

F. O. Woodruff: In the Doolittle and Woodruff method for insoluble extract much time is saved by a preliminary filtration through linen, after which the filtrate is warmed and passed through the tared paper, the insoluble residue being finally transferred to the tared filter and washed with repeated portions of hot water.

G. R. Stewart and *A. R. Mehrtens:* In this year's work on the coffee samples the Gorter method was found to be quite as satisfactory as before in regard to ease and rapidity of manipulation, but we believe that the results clearly show that the gravimetric determinations can not be taken as the final result in any case, owing to the impure caffein obtained. No good reason is therefore apparent for making this preliminary weighing, as it would be far more expeditious to transfer the chloroform extract directly to the Kjeldahl flask and proceed with the determination of the nitrogen. The results by the Lendrich and Nottbohm methods were, with one exception, obtained by a three hours' extraction with carbon tetrachlorid, as directed in the outline. The uniformly low amount of caffein extracted showed clearly that the three-hour period of extraction was insufficient. A six-hour extraction was therefore made on sample No. 4, Mocha coffee, and it will be observed that the result agrees closely with those obtained by the Gorter method. Unfortunately, lack of time prevented the repetition of the work on the entire series of coffees. It is, however, evident that either the time of extraction should be doubled or the amount of sample decreased to 10 grams, there being no reason why that amount should not be sufficient for an accurate determination.

The purity of the caffein extracted by this method is clearly shown by the results obtained. It is believed that further work is warranted by this latter modification if it is desired to perfect a method which will yield satisfactory gravimetric results.

ON TEA.

G. R. Stewart and *A. R. Mehrtens:* In the Doolittle and Woodruff method for insoluble leaf great difficulty was experienced in completing the determination in a reasonable length of time. Between two and three days were consumed in filtering and washing the residue. For the sake of accuracy it was thought necessary to use S & S No. 575 hardened filters. Even these papers were completely disintegrated on subsequent drying, so that a considerable error appears to have been introduced from the continued solvent action of the hot water. It does not seem, therefore, that the method is workable in its present form.

In both the Doolittle and Woodruff and the Dvorkovitsch methods for the determination of caffein in tea it was found that three extractions did not yield all the caffein present in solution. Five portions of chloroform, consisting of 50, 25, 25, 20, and 20 cc, respectively, were used in the Doolittle and Woodruff method, and five portions of chloroform consisting of 100, 100, 75, 75, and 75 cc, respectively, in the Dvorkovitsch method.

It is felt that the modified Stahlschmidt method is open to criticism on account of the appreciable error introduced by refluxing a measured volume from which an aliquot is afterwards taken, there being no way to absolutely prevent loss of water, which adheres to the condenser, stopper, and neck of flask. In other respects, facility of extraction, etc., this method was found to be a readily workable one.

RECOMMENDATIONS.

It is recommended—

(1) That the Gorter method for the determination of caffein in coffee be further studied with a view to adopting it as official. The particular line of work referred to is the comparison of the gravimetric determination of caffein with the result obtained by determining the nitrogen and multiplying by the factor 3.464, as it appears from the work here reported that the results by the gravimetric method are uniformly too high.

(2) That the Lendrich and Nottbohm method be further investigated. This further study would consist in modifying the method so as to use smaller samples—say, 10 grams instead of 20—and the time of extraction be increased, say, from 3 hours to 6 hours. The reason for further studying this method is because the purity of caffein extracted by the Lendrich and Nottbohm method is very clearly indicated by the results obtained.

(3) That the Doolittle and Woodruff method as given in Bulletin 105, page 49, should be modified, as great difficulty was experienced by the different analysts in filtering and washing the residue.

(4) That the Doolittle and Woodruff method and the Dvorkovitsch method for the determination of thein and caffein be further modified with reference to the number of extractions, as three extractions did not yield all the caffein present.

(5) That since the modified Stahlschmidt's method is open to criticism on account of the appreciable error introduced by refluxing a measured volume from which an aliquot is afterwards taken, a slight modification should be made to render this method readily workable.

(6) It is further recommended that no permanent changes be made in the methods until further study has been conducted along the lines indicated.

The following brief report was submitted by Mr. Mitchell at Mr. Barnard's request:

REPORT ON PRESERVATIVES.

By H. E. BARNARD, *Associate Referee.*

Early in the spring a circular letter was sent to each collaborating chemist requesting the following information:

(1) Are any new preservatives being used in foodstuffs, as is constantly reported to be the case by rival manufacturing interests?
(2) What preservatives are employed in foodstuffs?
(3) Is the method for the estimation of benzoic acid satisfactory?
(4) Is the method for the estimation of salicylic acid satisfactory?
(5) Is the method for the estimation of borax satisfactory?
(6) Do you consider saccharin to be a preservative?
(7) Is beta-naphthol used as a preservative, and is there a satisfactory method for its estimation?
(8) Are the fluorids used as a preservative and are there satisfactory methods for their detection?
(9) Are the peroxids used as preservatives and are there satisfactory methods for their detection?
(10) Along what lines can we best work?

From the responses to this inquiry, which was answered quite fully by each collaborator, I was able to tabulate the following data:

(1) The methods employed for the estimation of benzoic, salicylic, and boric acid are satisfactory.
(2) No new preservatives are being employed in the preservation of foodstuffs.
(3) Beta-naphthol is not employed in the preservation of foodstuffs.
(4) Saccharin, while not a true preservative, is, probably because of impurities present, of some value in inhibiting spoilage, and for that reason is classed by all the collaborators as a preservative.
(5) The methods for the estimation of saccharin are not satisfactory.
(6) Several collaborators express the opinion that the use of formic acid and its detection and estimation should be given attention.
(7) One collaborator suggests that nitrates and sodium silicate sometimes used as a substitute for borax in ice-cream cones, and calls atttention to the fact that methods for the examination of such products are not generally given.

These data were sent to all collaborators in August with the suggestion that their work should be directed to the development of definite methods for the detection and estimation of formic acid. Thus far no data have reached me, and it is not probable that any work of importance has yet been done along the lines suggested. In view of the facts developed through correspondence with collaborators and other food chemists, I am of the opinion that no more work is for the present necessary in the study of methods for the estimation of boric acid, salicylic acid, the fluorids, formaldehyde, and sulphurous acid. Investigations are still being continued with respect to benzoic acid and its salts, and the data should be reviewed by referees on the subject of food preservatives who may have charge of this work in the future.

REPORT ON WATER IN FOODS.

By HERMAN C. LYTHGOE, *Associate Referee.*

It has been found by different analysts that in many cases there is a darkening of the sulphuric acid in the vacuum method of determining water in food. This is true of substances other than spices. H. C. Gore has found that the acid is to some extent volatilized and is found in the sample, possibly causing some change in its composition. It is, therefore, recommended that the associate referee for 1912 make a study of the vacuum method, using different dehydrating agents.

METHODS OF ANALYSIS OF FRUIT PRODUCTS.

By A. E. Paul.

The ordinary examination of jams and similar fruit products includes the following determinations: Solids, soluble, insoluble, and nonsugar; sucrose; reducing sugars; glucose; polarization, direct, invert, and at 87° C.; ash and its examination; acids; soluble phosphoric acid; preservatives and color. In following strictly the methods described in Bulletin 107, Revised, separate portions must be weighed out for the following: Solids, insoluble solids, reducing sugars, polarization, and ash. Reducing sugars are determined in a solution clarified with lead subacetate solution, while the polarizations are carried out with the use of normal lead acetate. In both cases the reagent is added to a solution which in most cases contains considerable free acid. Normal lead acetate solution under these conditions is entirely unsatisfactory. On the other hand, adding the subacetate to such a solution amounts to neutralizing the acidity of the fruit product with the lead solution before the reagent has any effect. By first neutralizing with sodium hydroxid, only a few cubic centimeters of the lead solution will be required. Minimizing the amount of lead used minimizes the amount of potassium oxalate necessary for its removal. This is important, for the reason that in inverting with hydrochloric acid an amount of this reagent equivalent to the potassium oxalate present will be rendered practically inert, it being replaced by an equivalent of oxalic and acetic acids.

The method for total solids is very unsatisfactory, and the modifications described in the Proceedings of 1910 leave the matter in an unsettled condition.

No method for determining phosphates is described for the class of substances under consideration.

Applied to jelly powders which usually contain large percentages of gelatin, the methods for polarization and sugars are practically useless. All of these difficulties are overcome by proceeding as follows:

JAMS, MARMALADES, FRESH AND CANNED FRUITS.

Preparation of sample.—Unless the container is very large, reduce to a pulp the entire contents by grinding in a large mortar. If the container is too large stir contents thoroughly and grind a suitable quantity. Two portions of this are used in the analysis, one for the determination and examination of ash, the other for all of the other determinations.

Ash; ash soluble in water; ash insoluble in water; alkalinity of soluble ash; alkalinity of insoluble ash; ash insoluble in hydrochloric acid.—Determine according to the official methods on the same 5 to 10 gram sample.

Insoluble solids.—Weigh 50 grams, stir in previously boiled, warm, distilled water, very gradually at first, then more rapidly, transferring to a 250 cc measuring flask, finally filling to the mark. Let stand at least one hour, shaking frequently. When cold, again fill to mark. Strain through a fine muslin, returning liquid to flask. Aliquots of this solution are used for all the determinations described below.

Wash insoluble material repeatedly and thoroughly upon the cloth, transfer to an aluminum dish provided with a cover and dry to constant weight in a jacketed steam oven. In each case, cover the dish before removing from the oven. Weigh quickly. Discard the washings.

Soluble solids.—Calculate from specific gravity of solution from insoluble solids, at room temperature, according to the tables in Bulletin 107, Revised, pages 66 and 67.

Acids.—Place 50 cc of the solution from the insoluble solids in a 100 cc measuring flask. Add phenolphthalein and titrate with tenth-normal sodium hydroxid.

Polarization direct; polarization invert; polarization invert at 87° C.; sucrose; glucose; nonsugar solids.—To the neutralized solution from "acids" add slight excess of basic lead acetate solution, Bulletin 107, Revised, page 40, and a little alumina cream. Fill to the mark, shake, and filter. Then proceed according to the official methods.

Reducing sugars.—Determine in 3 cc of the solution used for "Polarization direct," by Munson and Walker, Bulletin 107, Revised, page 242.

Soluble phosphoric acid.—To 50 cc solution add 1 cc of 10 per cent sodium hydroxid solution, evaporate to dryness and ignite at a dull red until practically all carbon is consumed. Dissolve in dilute nitric acid, and proceed according to Bulletin 107, Revised, page 3, near top.

Preservatives.—Extract 50 cc of the solution according to Bulletin 107, Revised; weigh the dried residue and divide into two parts. To one add ferric chlorid; to the other apply Heide and Jacob's modification of Mohler's test.

If necessary, repeat according to the provisional method described in Bulletin 137, page 111.

JELLIES.

Prepare sample as directed for "Jams, etc.," omitting directions under "Insoluble solids."

FRUIT JUICES.

Proceed as in the case of "Jellies," using the undiluted material or a suitable dilution, depending upon the consistency of the product.

GELATIN MIXTURES, JELLY POWDERS, ETC.

Sucrose.—In a 100 cc flask dissolve the normal weight of the product in about 50 cc water. Dissolve in about 25 cc water an amount of pure tannic acid equal to twice that of gelatin present. Add to the solution in the flask. Fill to the mark and shake. Filter through a loose filter until 50 cc have been obtained. To this, in a 100 cc measuring flask add 3 cc of lead subacetate solution (Bul. 107, Rev., p. 40) for each 1 gram of tannin present, a little alumina cream, and water to make 100 cc. Shake and filter. Free from lead with potassium oxalate, filter; polarize and calculate sucrose according to Clerget.

Gelatin.—Determine nitrogen by the Kjeldahl or Gunning method and calculate to gelatin, using the factor 5.55.

The following committee reports were made by Mr. Tolman, as chairman.

Standardization of alcohol tables.—It will be recalled that the tables approved by the committee and by the Bureau of Standards were provisionally adopted by the association last year, more definite action having been deferred pending the decision of the committee on revision of the Pharmacopœia. (Bul. 137, p. 48.) No action having been taken by the latter committee in the year past, no further progress can be made by the committee of the association at the present time, and it is therefore recommended that the committee be discharged.

The report was received and the committee discharged as recommended.

Unification of methods of analysis of fats and oils.—It was found to be impossible to get a joint meeting of the various committees appointed on the standardization of methods by the different associations, but it is hoped that in 1912 at the meeting of the Eighth International Congress of Applied Chemistry such a joint meeting may be arranged by the collaboration of the sections of the congress, and the work be well organized. It is recommended, therefore, that the committee be continued.

The report was received and the committee continued as recommended.

As chairman of the committee on cooperation with other agricultural organizations, Mr. Wiley made the following report:

REPORT OF COMMITTEE ON COOPERATION WITH OTHER AGRICULTURAL ORGANIZATIONS.

By H. W. Wiley, *Chairman.*

The following resolution and agreement and the constitution proposed to effect the affiliation of societies interested in agricultural science are submitted for the information of this association:

A Proposed Affiliation of Societies Organized for the Advancement of Agricultural Science.

RESOLUTION AND AGREEMENT.

The authorized representatives of the associations and societies relating to agricultural science present at a meeting held in Washington, D. C., November 15, adopted the following resolution and constitution:

Resolved, That in order to promote common objects and interests there is special need of an affiliation of the various societies in North America which have for their objects the advancement of agriculture through scientific research:

That, therefore, the undersigned representatives of the Association of American Agricultural Colleges and Experiment Stations, American Association of Economic Entomologists, American Association of Farmers' Institute Workers, American Breeders' Association, American Phytopathological Society, American Society of Agronomy, American Society of Animal Nutrition, Association of Dairy Instructors, Association of Horticultural Inspectors, Association of Official Agricultural Chemists, Society for Horticultural Science, and Society for the Promotion of Agricultural Science hereby agree to affiliate under the following constitution, subject to ratification at the first regular session held by the societies mentioned subsequent to this date:

CONSTITUTION.

Article I. Name.

The name of this organization shall be the Affiliated Societies of Agricultural Science.

Article II. Purpose.

The purpose of the affiliation shall be to promote the common interests of the adhering societies, arrange periodically for a common place and time of meeting, promote economy and efficiency in publications, and otherwise to encourage cooperation in the advancement of agriculture through scientific research.

Article III. Membership.

The organization shall consist of national societies which have for their prime object the advancement of agriculture. The societies mentioned in the accompanying resolution shall be charter members. Additional societies may be admitted at any general meeting of the affiliated societies.

Article IV. Organization.

The general business of the organization shall be in charge of a council to consist of one member elected biennially by each adhering society.

The officers of the council shall be a president, a vice president, a secretary, and a treasurer, who shall serve for six months after their successors are elected. These officers shall constitute the officers of the organization.

Article V. Meetings.

A general meeting of the affiliated societies shall be held at least biennially, at a time and place to be determined by the council. Any adhering society may hold meetings at such times and places as it may select, but so far as practicable all should meet together biennially.

Article VI. Autonomy.

Each society shall retain its organization and shall have entire control of the election of its members and officers and all other matters not specifically delegated by it to the council of the affiliated societies.

Article VII. Annual dues.

Each society upon becoming a member of the organization shall pay to the treasurer of the council a pro rata sum, not to exceed $1 for each of its members, and a similar amount thereafter annually, the amount in each case to be fixed by the council. The fiscal year of the affiliated societies shall coincide with the calendar year.

Article VIII. Publications.

The Proceedings of the various societies may be issued individually, but all should conform to a uniform style of page, paper, and type, in order that they may constitute uniform parts of a set of Transactions of the Affiliated Societies.

The council may, upon the approval of the adhering societies, superintend the publication of their Proceedings and employ a general editor to cooperate with the editors of the various societies in securing uniformity, economy, and efficiency.

The council may arrange for the periodical publication of a journal of agricultural science, to contain reports in abstract of the meetings of the societies, brief notices, reviews, and contributions of general interest to the members of the affiliated societies and for the interchange of ideas on important problems of the day relating to agricultural science, this journal to be issued to members at a subscription price to be fixed by the council.

Article IX. Amendments.

Amendments to this constitution may be made at any general session of the affiliation upon the recommendation of the council, provided that 60 days' notice of the proposed amendments has been given to the president and secretary of the adhering societies.

Article X. By-laws.

The council shall formulate and adopt a set of by-laws to govern its actions under this constitution.

It will be seen that the proposed affiliation affects in no way the organization of the separate societies and would seem to be of value in unifying the work. Only one section of the constitution calls for special consideration, namely, Article VII, on annual dues. I recommend on behalf of the committee that the report be referred to the executive committee of the association, with power to act, except that they shall not enter into any financial agreement before reporting to the association at the next meeting.

The report was accepted and the recommendation adopted.

PRESIDENT'S ADDRESS.

By F. W. Woll.

The present convention is the twenty-eighth annual meeting which our association has held. As we look back upon the work accomplished in the past it is a source of great satisfaction to be able to feel that our association has been a most important factor in the development of agricultural chemical analysis and of agricultural science in this country. All who are conversant with the conditions as they existed in the early eighties and as they are to-day know that this statement is fully justified. Looking back to the former period, we find, in the words of our secretary, that "the few chemists who were engaged in agricultural research were acting in complete independence of each other in regard to methods of investigation and of research. Some of them were using the methods employed by German chemists, while others followed the instruction given by the French or English agricultural chemists. There was no unity of interest in the profession, nor any common system of work. The condition of analytical work may be truly described as chaotic. The result of such condition is easily imagined. There was no standard of comparison or reference. Buyers and sellers were continually wrangling over analyses which, made by different men following different methods, did not agree. The sellers' chemists uniformly obtained higher results than the buyers', and thus the door of litigation was constantly open.[1]"

The difference between the past and the present is marked all along the line. We can not point to one of the criticisms of the conditions as they existed that is not practically removed at the present time, and the credit for the progress made belongs largely to our association. It has been the central force that has made the progress and improvement in the methods of analysis and of research bearing on agricultural chemical problems, and in the relations of our agricultural chemists to each other and to their brethren engaged in industrial work. Hence we may have a pardonable pride at the contemplation of the work which our association has been able to accomplish within the relatively short period that it has been in existence and think with gratitude of the men who have been the leaders in this work and are largely responsible for the progress that has been made. Of the small band of agricultural chemists who were prime movers in the organization of this association, a large majority have now gone to their reward and but few are still actively engaged in the work to which they have devoted the best efforts and energies of their lives. Most of the present membership of our association belongs to the second generation, who received their college or other preparatory technical education during the first decade or two after this association was organized. As they may not, therefore, be especially familiar with the early history of the association a few words along this line may not be amiss.

Although our association officially dates back to 1884, when the organization was effected and the constitution adopted, there had been held four meetings prior to that date, by eastern or southern chemists interested in the study and further development of methods of fertilizer analysis. The first meeting was held in Washington, 1880, in response to a call signed by the Hon. J. T. Henderson, commissioner of agriculture of Georgia, and issued at the request of former Director Redding, of Georgia Experiment Station, while the following meetings were held in Boston (also in 1880), in Cincinnati in 1881, and in Atlanta in 1884. Out of the Atlanta meeting grew the organization of our present association at the meeting held in Philadelphia, September 8 to 9, 1884.

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[1] U. S. Dept. Agr., Division of Chemistry Bul. 57, p. 16.

The objects of the association, according to the constitution adopted at that meeting, were twofold—(1) to secure as far as possible uniformity in legislation with regard to the regulation of the sale of commercial fertilizers in the different States, and (2) uniformity and accuracy in the methods and results of fertilizer analyses. It soon became clear to all that the primary usefulness of the association lay in the direction of studies and comparisons of methods of analysis rather than in influencing legislation with regard to the regulation of the sale of commercial fertilizers in different States, and during the past quarter of a century the work of the association has been almost wholly in the former direction. The main and about the only direct result accomplished in a legislative line was the adoption of the report of the special committee with regard to a uniform fertilizer law in 1898.

The association was organized for the purpose of studying methods of analysis of phosphoric acid, nitrogen, and potash, but its work was only confined within these narrow limits for two years. As early as 1886 Commissioner of Agriculture Norman J. Colman, in a letter to the executive committee, suggested that it would be both wise and profitable to widen the scope of the investigations conducted to include other subjects than those immediately connected with the analysis of fertilizers, and the president of the association that year, our present secretary, in his presidential address likewise urged an extension of the character of the work to be undertaken. "Every problem," he says, "connected with chemical agricultural analysis falling within the range of the studies of anyone connected officially with the agricultural interests of the country seems to me to be a proper theme for our discussion here. Thus all the problems relating to the adulteration of food, the general method of agricultural analysis, and all other matters which concern, in common, the analyst and the public, could be included among the duties and privileges of this body."

Other subjects have as a result been added to the list of materials for which methods of analysis were to be studied and discussed by our association. First came cattle foods and dairy products in 1886; then, in 1887, fermented liquors and sugar; 1890, soils and ash; 1894, tannin; 1897, liquor and food adulteration; 1898, insecticides; 1901, food adulteration (with 17 associate referees, which by this time has been increased to 30); 1903, medicinal plants and drugs; 1904, inorganic plant constituents; and in 1908, water, until at the present time it may be truthfully said that our work more than covers the entire field of agricultural chemical analysis as outlined by our secretary 25 years ago.

Our association is unique in several ways: It is the largest body of agricultural chemists in the world and has a wider scope than any of the foreign societies organized for similar work. It has been its good fortune to have the cordial support of our National and State agricultural institutions of research and instruction, and through the cooperation of the United States Department of Agriculture our worthy secretary has been able to serve the association as president, member of the executive committee, and in his present capacity, for an unbroken series of 28 years. The history of the Association of Official Agricultural Chemists, with Dr. Wiley left out, would be like Shakespeare's Hamlet without "the melancholy Dane," and I imagine that when his (Wiley's) labors are ended and his biographers pass in review the story of what he accomplished, they will accord a very prominent place to his faithful and efficient connection with our association; and let it be a consolation and satisfaction to him to know, amid all abuse, ridicule, and misrepresentation from special interests, from whose adulterated products of all kinds he has protected God's poor, the patient and long-suffering public, that his work in our association and in the interest of pure food, drink, and drugs is duly appreciated by our members and by the general public through the length and breadth of our land. They know, as we know, that he has fought their fight courageously, honestly, and with unflinching fidelity. [Applause.]

Our association is unique in another way. By our laws of organization and established custom we have now a staff of over 50 referees and associate referees who each year plan cooperative work in their respective lines and prepare reports on the work connected with analyses of special substances. Each one contributes what he can toward the study and perfection of these methods or works out new methods by which our knowledge of the chemical analysis of materials coming within the range of the association may be enlarged and greater accuracy of results obtained. This splendid permanent organization for work which we thus possess makes a most valuable asset and has been of the greatest benefit to the chemists of our country, as well as to the referees and the associate referees themselves and their collaborators. It makes the work of the officers of the association in preparing the programme of the convention largely perfunctory. There is probably no set of officials in a national organization of the importance or membership of this association who have an easier task in this direction than have your officers. No solicitation of papers here or urging attendance or participation in discussions. The president calls the meeting to order and appoints a few committees, and the referees and associate referees do the rest.

It is easily seen that this organization of the work of the association has its weaknesses as well as its advantages. The mere fact that so many take part in the programme may dampen the ardor of some, and there is also a danger that the similarity of the reports presented and the somewhat stereotyped plan of cooperative work undertaken in many lines may tend to lessen the interest of some members in the work of the association and in the proceedings of our convention. The extreme specialization which is inevitable under present-day conditions also renders it difficult for some chemists to follow with profit the reports and discussions in fields of chemical analysis far removed from those in which their own special work generally lies.

There can be no doubt, however, that participation in the cooperative work of the association offers an excellent opportunity for study of special methods and for comparing one's own results and working methods with those of other chemists. Many an error of analysis has been discovered in the past, and faulty methods of manipulation have been corrected, through the check furnished by the collaboration of chemists working with samples of the same origin and under the same specific directions. One suggestion may be made in this connection, that referees arrange to have a sufficient number of copies of the compilation of the results obtained in the year's work to supply them by mail to all chemists who have cooperated with them, thereby giving those who are not so favored by geographical location or a good-sized pocketbook that they are able to attend the meetings regularly, an opportunity to find out, while the work is fresh in their minds, how their results compare with those of other chemists. Another advantage would be that chemists could then go over the work again early in the fall in case it should prove desirable to do so, before instructional or research work once more demands all their time and energies.

A suggestion may be in order with reference to the cooperative work planned. Referees sometimes forget that most collaborators are busy men, whose working hours are wholly taken up with the work for which they receive their modest allowance, and more cooperative work is often requested than can be done by a chemist during a period of several weeks. The result is that many are discouraged from taking part in the work at all. If each referee asked for cooperation with regard to only a couple of points, more chemists would feel disposed to undertake the work than is now often the case, and the returns would be of more value both to the referee and to the association. A small step in advance along the whole line each year will bring more satisfactory results, and we shall progress faster and farther than if too much is attempted. If referees and associate referees on the respective subjects will confer carefully beforehand regarding the points to be studied and bear in mind the suggestion just made, there will not be much occasion for apologies in the reports on cooperative work presented, and each year will see decided progress made toward more satisfactory methods of analysis.

Active participation in the work of the association is not only important and an inspiration to the individual chemists who plan or carry it through—the institutions they represent and their directors are benefited to a similar extent. During the last 12 years the chemical control work that our institutions are called upon to do has widened in scope, and nearly all now have duties along one or more lines of inspection. The fertilizer control work, as you all know, came first, then came feeding stuffs, and of late insecticides and fungicides have been added to the list, so that most of us may now be called upon at any time to prove the correctness of the analytical work done with these materials, either before the courts or to our friends the commercial chemists, or those employed by manufacturing firms.

While we all feel the responsibility of our position, and do not report results until we have full confidence in their correctness, mistakes will occasionally occur in spite of all care, and the annual checking up of methods and results afforded by the cooperative work of this association here comes in as a comfort and an aid. We find our own results corroborated by chemists working with the same materials but under widely differing conditions, often with minor differences in regard to details of manipulation, and with different standard solutions, chemicals, balances, and weights. Our confidence in the work of our laboratory and in ourselves is thus strengthened, or, if errors are discovered, we are led to look for and correct them, with a very pleasant feeling of satisfaction resulting. For this reason it would seem we can well afford to give up a week or more, if necessary, of our own or our men's time each year to do the cooperative work planned along the lines of activities in which our laboratories are directly interested. It is good for our chemists, it is good for our institutions, and it is good for our association, whose work is advanced nearly in proportion to the number of chemists cooperating under the guidance of able referees and associate referees who are specialists in their respective lines of study.

The danger of overspecialization is with us always, in chemistry as elsewhere, and those responsible for the training of young chemists can not emphasize too strongly the necessity of a thorough fundamental knowledge of general chemistry and chemical analysis before specialties are taken up. The broader and more solid foundation the chemist brings to his chosen work the better position he will be in to grapple with the problems which this presents, the more valuable his services will prove to his employers, and the better are his chances of rising above the level of the average, in reputation and in the emoluments a useful man can command. In addition to a thorough fundamental training the cardinal virtues of care, honesty, and accuracy in the conduct of one's work are primary requisites, without which no chemist can hope to win recognition.

The story is told of Bunsen that while making a quantitative analysis of some material at one time he was unfortunate enough to upset the beaker in which the solution was heated, and spilled some of it on the laboratory table. With the ever-present German laboratory sponge he wiped the table and squeezed the sponge over the beaker. A similar mishap occurred again a little later and the difficulty was remedied in the same way, the famous chemist mumbling to himself as he squeezed the solution into the beaker the second time, "Oh, pshaw! if that happens once more I shall have to begin all over again." The story, if true, only illustrates that a man may be a great scientist and discoverer without being a good analyst. For the large majority of us who are not by nature endowed with marked ability to discover new paths in science, or to enlarge in a brilliant way the sum of human knowledge, the safer plan, by far, is to do well what we can do, so that our results will bear the severest scrutiny and we may have the satisfaction of knowing that they can be corroborated by carefully trained chemists everywhere, irrespective of geographical location, nationality, or position.

As suggested at the outset, the work of the association in the past has been of the greatest service to the chemists of our country and elsewhere, as well as to our agricultural interests in general. Our methods of analysis have been closely studied, and those proving the most accurate and satisfactory have been given official stamp after continued careful investigation and scrutiny. Much progress has thus been made, and, while the best of to-day may not stand the test of to-morrow or the day after, we believe that along most lines of analytical work in which we are interested the methods now at our disposal will stand the test of time and will require further study only in regard to details of manipulation. Along other lines, however, much still remains to be done before we can feel that this goal has been reached, and in order to reach it we shall need the hearty cooperation and the best assistance of all members in their respective fields of study. Our experience in the past and the character of our present large membership justify the confident hope that the importance of our association and its usefulness to the chemical profession and to agricultural science will prove still greater in the future than has been the case in the past, and that it will receive the cordial support of all members in solving the problems confronting it now and in the future, the work being characterized by the same spirit, enthusiasm, and energy as have overcome difficulties and aided us in gaining a clearer knowledge in the past.

TUESDAY—AFTERNOON SESSION.

REPORT ON THE SEPARATION OF NITROGENOUS BODIES (MEAT PROTEIDS).

By C. R. Moulton, *Referee.*[1]

The cooperative work done this year on the separation of meat proteids has been limited to the determination of total nitrogen in concentrated beef extracts. The lack of agreement among the analysts in this work during the past two years, on the one hand, and, on the other hand, the contention by some that the official methods are satisfactory led the referee to make a further test of the methods for total nitrogen.

The samples of concentrated beef extract employed were the same as those used in our previous work,[2] and were prepared in the laboratories of the Missouri agricultural experiment station. The samples showed a large amount of crystalline bodies which settled out on standing. It was suggested that, in order to remove any errors which might arise from this source, the sample be very thoroughly mixed and a large sample be weighed out by the method of difference, dissolved in water, filtered, and made up to a volume. Aliquots for total (soluble) nitrogen could be drawn from this solution. This, of course, gives soluble nitrogen, but it affords a good comparison for the method for total nitrogen and removes any error due to lack of uniformity of sample. Letters were sent to 22 laboratories, and 7 men signified their ability to cooperate.

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[1] Presented by P. F. Trowbridge.

[2] Sample 1 was sent to J. T. Willard, E. B. Forbes, A. D. Emmett, Paul Rudnick, and A. Lowenstein. Sample 2 was sent to T. C. Trescot and T. L. Haecker.

REPORTS OF REFEREES.

J. T. Willard reports the work of J. W. Calvin as follows:

Nitrogen determination.—Two charges were weighed out as directed, dissolved in water, and made up to volumes of 250 cc. Two portions of 25 cc each, or one-tenth of the charge, were taken from each solution and digested with 0.7 gram of mercury and 30 cc of sulphuric acid for three and one-half hours, although the liquid was colorless after the end of the first hour. The following results were obtained: Charge 1: 6.7050 grams, aliquot 0.6705; per cent nitrogen, 8.755, 8,725. Charge 2: 7.2782 grams, aliquot 0.7278; per cent nitrogen, 8.759, 8.759.

R. C. Collison reports upon the sample sent to E. B. Forbes as follows:

About 10 grams of the well-mixed sample were weighed by difference into a Kjeldahl flask; 50 cc of sulphuric acid and 0.6 gram of metallic mercury were added. The contents were digested for about one hour or one and one-half hours and 10 grams of potassium sulphate added. The digestion was continued until the solutions were practically clear. Total time of digestion, six hours. The solution was then made up in a volumetric flask and an aliquot distilled in the usual way. Results: 8.74, 8.73, 8.75, 8.80; average, 8.755 per cent.

Another sample of about 10 grams was dissolved in water made up in a volumetric flask and an aliquot digested as before, using 20 cc of sulphuric acid, 0.6 gram of mercury, and 10 grams of potassium sulphate. The whole aliquot was distilled. Percentage results on nitrogen by this method: 8.74, 8.80; average, 8.77 per cent.

A. D. Emmett reports as follows:

The sample was digested at a low temperature until the frothing ceased. This generally occupied from 15 to 20 minutes. The 5 grams of potassium sulphate and 0.65 grams of mercury were then added. The digestion was continued until the mass became clear, which took varying lengths of time, as shown in the table. The heat was turned off as soon as the digest became clear, and the neck of the flask was then washed down, after which the digestion was continued for one and one-half hours. Then 250 cc of ammonia-free water were added, a small amount of pumice, and the customary amount of potassium sulphid and sodium hydrate. The distillation was carried out so that 250 cc of the distillate came over in about 35 to 40 minutes. The indicator used was Congo Red, the standard acid was hydrochloric, and the alkali, sodium hydroxid. Blank determinations were run on all reagents and the correction applied for these in making the final calculations. We made the determinations on both unfiltered and filtered samples. The data seem to indicate that with the exception of test "A" one can obtain fairly satisfactory results by the method just outlined, both for the filtered and unfiltered samples.

Nitrogen results by A. D. Emmett on filtered and unfiltered samples.

Test number.	Total amount taken.	Total volume.	Volume taken.	Time of digestion.			Nitrogen.	
				Until clear.	After clear until end.	Total.	Unfiltered.	Filtered.
	Grams.	*cc.*	*cc.*	*Hr. min.*	*Hr. min.*	*Hr. min.*	*Per cent.*	*Per cent.*
A 1	10.1044	250	25	0 55	1 30	2 25	8.80	8.41
2							9.04	8.67
3							8.80	8.22
Average							8.87	8.43
B 1	9.4593	250	25	1 15	1 30	2 45	8.65	8.54
2							8.65	8.47
3							8.65	8.46
Average							8.65	8.49
C 1	7.6605	250	25	1 05	1 30	2 35	8.55	8.50
2							....	8.53
3							8.65	8.43
Average							8.60	8.49
D 1	5.4064	250	25	0 50	1 30	2 20	8.65	8.55
2							8.67	8.50
Average							8.66	8.53

Paul Rudnick makes the following report:

We find in this laboratory that the quantity of extract to be digested must not exceed 0.85 gram, and should probably be less than this in order to get concordant results; the time of digestion is fixed at two hours after the digestion comes to final color, and never less than three hours in all, and the method employed is the so-called combination Kjeldahl-Gunning method, using 5 grams of potassium sulphate and 0.5 gram of metallic mercury.

Nitrogen results reported by Paul Rudnick.

Solution.	Weight of sample.	Time of digestion.	Nitrogen.
	Grams.	*Hours.*	*Per cent.*
50 cc:			
1A	0.9764	3	9.22
1B	.9764	3	9.25
2A	.9914	3	8.97
2B	.9914	3	8.99
3A	1.0226	3	8.87
3B	1.0226	3	8.89
100 cc:			
1C	1.9527	3	9.27
2C	1.9828	3	8.97
3C	2.0452	3	8.92
1, 2, 3, D	1.9957	3	8.71
1, 2, 3, E	1.9957	3	8.97
4A	3.8428	5	8.64
5A	4.4015	5	8.13
150 cc:			
4B	5.7643	9	8.61
5B	6.6022	9	8.24

The solutions were made up in accordance with your suggestion to weigh approximately 10 grams by difference, dissolve in water, filter, and make up to volume. The weights were taken and the solutions made up as follows:

Description of solutions.

Solution No.	Beef extract.	Volume of finished solution.
	Grams.	*cc.*
1.........	9.7635	500
2.........	9.9140	500
3.........	10.2262	500
4.........	9.6071	250
5.........	11.0037	250

In the table of results the figures in the first column refer to the number of the solution, while the letters serve to distinguish the successive aliquot portions from each solution. The results marked 1, 2, 3, D, and 1, 2, 3, E, respectively, were drawn from composite solutions made up from equal volumes of solutions 1, 2, and 3.

Nitrogen was determined in all cases by the so-called Kjeldahl-Gunning method, using 5 grams of potassium sulphate, 0.5 gram of metallic mercury, and 25 cc of sulphuric acid. Blanks were deducted in each case as usual, amounting in this case to 0.0007 gram of nitrogen, and the results reported are those obtained after correcting for this blank.

The results show clearly the tendency toward lower results, with larger samples, in spite of increased time of digestion. It seems highly probable that this is due to the same cause which produces the tendency toward low results in commercial ammoniates of high nitrogen content, such as dried blood, tankage, etc., when the time of digestion is too short. Whether this is due to the formation of organic amins or amino acids, etc., which do not react with the indicator as equivalents of ammonia is an interesting question. So far as I know, it is commonly assumed among analysts that the oxidation of the nitrogen compounds in this determination proceeds quantitatively until all the nitrogen is present as ammonium salts, but it does not seem to me that this view can be correct.

It may be of interest to you to know the results obtained on determining nitrogen in the papers through which the above solutions were filtered:

Nitrogen found in filter papers used in preceding determinations. (*Rudnick.*)

Solution.	Weight of sample.	Nitrogen.
	Grams.	*Per cent.*
1.......	9.7635	0.10
2.......	9.914	.08
3.......	10.2262	.07
4.......	9.6071	.07
5.......	11.0037	.06

The same sample was analyzed in the Missouri laboratories by E. E. Vanatta. The Kjeldahl-Gunning method was employed, using 30 to 35 cc of sulphuric acid and 0.7 gram of metallic mercury. This was digested until the sample was no longer "pasty," when from 5 to 7 grams of potassium sulphate were added. The digestion was continued until the sample was colorless, when the neck of the flask was washed down with water and the digestion was continued. The aliquots given in the table were used for digestion. Twenty-five grams were dissolved in water, filtered, and made up to 1,000 cc.

Report on sample 1, by E. E. Vanatta.

Solution and aliquot.	Hydrochloric acid.	Nitrogen.	Weight sample.	Time.		
				Before clearing.	After clearing.	Total.
10 cubic centimeters:	cc.	Per cent.	Grams.	Min.	Min.	Hr. min.
A 1a	16.67	8.362	0.25	25	30	0 55
b	Lost.				30	0 55
2a	16.77	8.412			60	1 25
b	16.88	8.467			60	1 25
3a	16.67	8.362			120	2 25
b	Lost.				120	2 25
20 cubic centimeters:						
B 1a	33.45	8.389	0.50	25	30	0 55
b	Lost.				30	0 55
2a	33.43	8.384			60	1 25
b	33.55	8.414			60	1 25
3a	33.60	8.427			120	2 25
b	33.53	8.409			120	2 25
35 cubic centimeters:						
C 1a	58.30	8.355	0.875	35	30	1 5
b	58.75	8.420			30	1 5
2a	58.85	8.434			60	1 35
b	58.75	8.420			60	1 35
3a	59.05	8.463			120	2 35
b	59.03	8.460			120	2 35
100 cubic centimeters:						
D 1a	167.28	8.391	2.500	55	30	1 25
b	Lost.				30	1 25
2a	171.12	8.583			60	1 55
b	171.48	8.601			60	1 55
3a	169.83	8.519			120	2 55
b	171.53	8.604			120	2 55
Average		8.444				

Mr. Lowenstein writes as follows in regard to this work:

In this connection we were very much interested in the report which you submitted to the association, as printed in Bulletin 137, Bureau of Chemistry. We note that you state that you had "some difficulty in getting good duplicate results for nitrogen in the case of beef extracts when samples of about 2 grams were weighed out for total nitrogen and digested by the modified Kjeldahl method." We have used this method in our laboratory for a long time and have never experienced any such difficulty as you mention, even when the work was done by men who have had very little experience in analytical work. In the attached analysis Analyst B has had only two months' experience in commercial analytical work. We could cite numerous instances of analyses run by this method, in all of which our duplicate results have been as close as in the attached, and we know of no instances where we have had any such results as appear in your report.

We thought at first that your trouble might have been due to the sample, as in our ordinary run of commercial work we very rarely run across a sample of beef extract such as submitted by you. However, we have been able to get good duplicate results from the sample submitted within our own laboratory. We will be very much interested to see the cooperative results on this sample.

The sample submitted was run by the method suggested in your letter and also by the modified Kjeldahl method, two grams of the sample being used for the determination.

Nitrogen results on official sample by two methods.

Modified Kjeldahl method.		Moulton method.	
Analyst A.	Analyst B.	Analyst A.	Analyst B.
Per cent.	Per cent.	Per cent.	Per cent.
8.98	8.92	8.98	8.89
8.90	8.90	8.99	8.85

Our method of digesting the sample, in detail, is as follows:

Two grams of the sample are weighed in a tared filter paper and the entire sample is put in a Kjeldahl flask. One-half gram of metallic mercury and 10 grams of crystalline potassium sulphate are then added together with 25 cc of concentrated sulphuric acid. The sample is then digested for three hours, after which with the addition of a little zinc dust and a few drops of paraffin oil and 350 cc of water, together with 50 cc of caustic soda solution containing the proper amount of potassium sulphid, it is distilled into a 500 cc Erlenmeyer flask containing 25 cc of standard hydrochloric acid (1 cc equals 1 per cent ammonia where a 1 gram sample is employed).

The analytical work on this sample was done by J. J. Vollertsen and an assistant, to whom credit is due.

On sample 2 T. C. Trescot reports as follows:

Results on sample 2 by the Gunning method, varying size of sample.

Weight of sample.	Total nitrogen.
Grams.	*Per cent.*
1.7382	8.73
1.2974	8.71
1.9235	8.66
1.9702	8.77
0.2395	8.67

The digestion was continued four hours. This is the usual method for determining nitrogen in meat extract in this laboratory. Following the suggestion of the referee, we dissolved 20 grams of the extract in 200 cc of water, filtered and determined the nitrogen in 10 cc portions of the filtrate. The results were as follows: Weight of sample, 1 gram; per cent of soluble nitrogen, 8.28 and 8.22.

In order to check the work upon the referee's sample, we determined the nitrogen in a sample of Liebig's extract with the following results: Weight of sample, 1.8820 grams; per cent of nitrogen, 9.54; 0.9578 gram, 9.55 per cent; 0.9578 gram, 9.49 per cent, and 0.9578 gram sample, 9.49 per cent nitrogen. In this work the time required for a sample to become clear on digestion was by no means constant. The length of time required depended largely upon the size of the sample.

The size of the sample seemed to have no influence upon the percentage of nitrogen.

T. L. Haecker reports the work from the Minnesota laboratory by Mr. Anthony as follows:

Nitrogen results on referee's samples as reported by Haecker.

Beef extract samples.	Weight of sample.	Aliquot parts taken for digestion.	Total time of digestion.	Hydrochloric acid used.	Soluble nitrogen.	Remarks.
	Grams.	*cc.*	*Hours.*	*cc.*	*Per cent.*	
1	12.8864					Dissolved in H_2O and made to 500 cc.
1-a		50	5½	33.89	8.641	Digested with K_2SO_4 and HgO.
1-b		50	5½	33.81	8.621	Do.
Average					8.631	
1-c		50	5½	33.82	8.623	Digested with HgO only.
1-d		50	5½	34.07	8.687	Do.
Average					8.655	
1-e		10	5	6.70	8.542	Digested after Milbaur's method.
1-f		10	5½	6.69	8.530	Do.
Average					8.536	

Nitrogen results on referee's samples as reported by Haecker—Continued.

Beef extract samples.	Weight of sample.	Aliquot parts taken for digestion.	Total time of digestion.	Hydrochloric acid used.	Soluble nitrogen.	Remarks.
	Grams.	*cc.*	*Hours.*	*cc.*	*Per cent.*	
2	5.4521	8				After digestion made to 500 cc, digested with K_2SO_4 and HgO.
2-a			50	14.28	[1] 8.605	
2-b			50	14.33	8.636	
Average					8.62	
3	4.4171	8				Do.
3-a			50	11.29	[1] 8.398	
3-b			50	11.23	8.353	
Average					8.375	

[1] Total nitrogen.

Comments on this work made by Mr. Haecker are as follows:

From the thoroughly mixed substance three individual samples were weighed out by the method of difference.

Sample No. 1, weighing 12.8864 grams, was dissolved in a beaker with water, filtered, and made up to the volume of 500 cc at 15° C. From this solution, four aliquot parts of 50 cc each (1-a, 1-b, 1-c, 1-d) were drawn for digestion. To samples 1-a and 1-b 10 grams of potassium sulphate and about 0.7 gram of mercuric oxid were added; to samples 1-c and 1-d only about 0.7 gram of mercuric oxid.

The digestion was carried on, first, with a low flame until most of the water was evaporated and the sulphuric acid fumes began to rise, then potassium sulphate and mercuric oxid were added and the digestion carried on *for four hours after the solution became colorless.* The distillation of the samples was carried out as usual, with the only difference that the boiling was slow and the whole process of distillation continued for about one hour and a half. An excess of standard hydrochloric acid was used to catch the distillate, and a corresponding potassium hydroxid solution with cochineal as an indicator was used for titration.

Two blanks were run with about 1 gram of chemically pure anhydrous lactose ($C_{12}H_{22}O_{11}$) using the modified Gunning method for digestion. The correction of 0.5 cc of hydrochloric acid used was equally applicable to all analyses.

Sample No. 2, weighing 5.4521 grams, and sample No. 3, weighing 4.4171 grams, were placed in Kjeldahl flasks and digested after the modified Kjeldahl-Gunning method. The heating of the substance was slow, and the digestion was continued for *six hours after the substance became colorless.* The digested samples were made up to 500 cc at 15° C., and 50 cc duplicates were drawn for distillation. The distillation was carried on as with sample No. 1.

The results obtained by either method show a marked check in the duplicate analyses and between each other. A discrepancy of only 0.25 per cent occurs between sample No. 2 and sample No. 3. This discrepancy I think can be credited to an analytical error or to the fact that the last sample did not correspond to the average, because of the accumulation of small crystals in the bottom of the weighing glass.

Discrepancies in the nitrogen content of a larger and a smaller sample should be credited only to an incomplete digestion or to an error in the process of distillation. Larger samples correspond better to the average than small samples. Even if duplicate small samples of 0.5 gram would check closely they do not give us a guarantee of an average result. Larger samples, however, check equally well, and the results come the nearest to the average composition of the material.

The distillates obtained were clear and no difficulty with the indicator could be noticed in the titration.

To test whether the amido nitrogen is completely hydrolyzed by the common Kjeldahl method Milbaur's modification as applied to hydrazones and osazones was used. The two results obtained check exceptionally well, but, of course, from only two determinations no conclusions can be drawn.

The method of Milbaur was used in the following way: 10 cc of the liquid sample were measured from a burette into a Kjeldahl flask; 40 cc of water and 50 cc of concentrated sulphuric acid were added; 3 grams of powdered chemically pure zinc previously washed in 1 per cent sulphuric acid were poured into the flask. The contents were previously cooled to prevent a too strong reaction. The substance was heated very slowly over a wire gauze, and the heating continued until no more hydrogen was generated. The generation of hydrogen ceased after one-half hour and then two drops of metallic mercury were added and the digestion carried on as usual. After the substance became white it was left to cool a little, and 1 gram of chemically pure potassium dichromate was added and the digestion carried on for two hours longer. Milbaur uses 2 grams of potassium persulphate, but since this was not at hand the dichromate was used instead. The solution was distilled from a copper flask to prevent bumping. [In this connection the referee wishes to call attention to results obtained two years ago in the Missouri laboratory on the same sample—total nitrogen on 2-gram samples, 9.341 and 9.474 per cent; soluble nitrogen on 0.5-gram samples, 8.389 and 8.731 per cent.]

DISCUSSION.

A study of the foregoing data shows rather marked uniformity in the results on total nitrogen. On sample No. 1 the three investigators report results between 8.7 and 8.8 per cent of total nitrogen. Good duplicates are obtained by different chemists. Two of the investigators get rather higher results, averaging rather close to 9 per cent. Comparing insoluble nitrogen in this sample, the figures submitted by Mr. Emmett are exceedingly good duplicates of the figures obtained in the Missouri laboratory. In connection with the higher results obtained by the two collaborators in the stock yards, the following extract from Mr. Rudnick's report is of interest: "I should like to comment upon the data obtained in the determination of nitrogen on the water-insoluble residue remaining on the filters. These filters were well washed and no trace of yellow color was visible on them. It would seem, therefore, that the crystalline water-insoluble substances may possibly contain crystalline meat bases or other nitrogenous organic compounds in addition to inorganic solids. I regret that lack of material and time did not permit a further investigation of this question, which might throw some light on the discrepancies in the determination of nitrogen in beef extracts."

At the Missouri station we had no trouble in getting crystalline bodies into solution provided the sample was allowed to stand covered with water for 15 or 20 minutes before filtering. The residue on the filter papers in filtering out the insoluble nitrogen had the appearance of a brownish coagulum and no crystals were left.

Turning our attention to sample No. 2, it is seen that the two collaborators obtained results for total nitrogen that agreed very well. Their results on soluble nitrogen do not show the same agreement, but this may be accounted for by insufficient oxidation or some other error in the method. In this work the collaborators used almost exclusively the so-called Kjeldahl-Gunning method. Following the referee's suggestion that the best method be selected, they have, with two exceptions, used this method. It therefore seems to the referee that when care is taken to get a uniform sample and when from 0.2 to 2.5 grams of the substance are digested by the Kjeldahl-Gunning method, allowing four hours for the digestion, uniform results can be obtained for total nitrogen.

RECOMMENDATIONS.

The referee has concluded as a result of this work that the contention of W. D. Bigelow and F. C. Cook, that the present method for total nitrogen is efficient, holds when care is taken that a well-mixed and uniform sample be used for analysis and that the oxidation be complete. To secure complete oxidation the cooperators choose generally a combined Kjeldahl-Gunning method. In this connection the referee wishes to call attention to the report of committee A on recommendations of referees for 1907. Under "(1) Nitrogen," recommendation 5, page 129, Bulletin 116, there is a recommendation that a special method be introduced combining the features of the two methods—the Kjeldahl and the Gunning. The referee finds no further action on this important point. He would therefore recommend—

(1) That on page 7, Bulletin 107, Revised, between "(b) Gunning method" and "(c) Kjeldahl method modified" there be inserted a Kjeldahl-Gunning method as follows:

(b) KJELDAHL-GUNNING METHOD.

Place the substance to be analyzed in a digestion flask, employing from 0.7 to 3.5 grams, according to its proportion of nitrogen. Add 0.7 gram of mercuric oxid, or its equivalent in metallic mercury, and from 20 to 30 cc of sulphuric acid. Start the digestion and, after frothing has ceased and contents of flask are no longer pasty, add from 7 to 10 grams powdered potassium sulphate. When digest is colorless cool and wash down the neck of the flask with water and continue the digestion till oxidation is complete. Finish as in the Kjeldahl method, omitting potassium permanganate.

(2) That in 7 (a), line 1, same reference, page 108, the words "or Kjeldahl-Gunning method" be inserted after "the Gunning method."

(3) That in the same bulletin, page 114, 7 (a) be made to read: "Employ the Kjeldahl-Gunning method, page 7, under 'I. Fertilizers.' Use from 0.2 to 2.0 grams of thoroughly mixed sample and digest for at least four hours."

(4) That the work for next year be a continuance of the separation of the nitrogenous bodies (meat proteins).

NOTES ON THE DETERMINATION AND VALUATION OF POTASH.

By P. F. TROWBRIDGE.

Many claims are being made on the part of certain fertilizer manufacturers as to the great advantage of manure concentrate or manure ashes, not only as to the plant food present, but as to the good effect upon the land due to the alkalinity of the material. The same manufacturers have difficulty in making the potash content of the fertilizer run uniform and requests are repeatedly received that the analysis of potash be modified to include total potash.

This can not be done according to the present fertilizer law of Missouri, which specifies that the potash must be soluble in water. In order to get some information at first hand the writer himself secured directly from the furnaces a sample of the ashes from burned manure and also a sample of ashes from a limekiln in the State. Examination of the alkalinity of these ashes shows that the manure ashes have an equivalent of 30.38 per cent of calcium oxid, while the wood ashes have an equivalent of 53.73 per cent.

Samples of these ashes were boiled for several hours with distilled water and thoroughly washed to separate the water-soluble potash as in the regular fertilizer analyses. The insoluble residue was then examined for total potash by the J. Lawrence Smith method to determine the amount of insoluble potash. The following tabulation shows the results obtained:

Potash content of manure and wood ashes.

Determinations.	Manure ashes.	Wood ashes.
	Per cent.	*Per cent.*
Soluble potash	5.916	4.670
Insoluble potash	7.702	.938
Total	13.618	5.608

It is quite probable that the large amount of dirt in the manure may have formed with the burned ashes an insoluble silicate, which would account for the large amount of insoluble potash.

An examination of five samples of ash from the leaves of the peach shows it to contain 26.588 per cent of soluble potash and 3.239 per cent of insoluble potash. The ash from several samples of timothy hay contains as high as 30 per cent soluble potash in the hay cut in the earlier stages of growth, while in the ash from the ripe hay the soluble potash is as low as 23 per cent. In these samples of ash from timothy hay only about 1 per cent of insoluble potash is found.

REPORT ON THE SEPARATION OF NITROGENOUS BODIES (MILK AND CHEESE).

By A. W. BOSWORTH, *Associate Referee*, and O. B. WINTER.

PLAN OF WORK.

As no recommendations were made for this line of work by the association last year, the referee has selected for study two of the newer methods which have been published recently. These methods are the Folin[1] method for the determination of ammonia and the Van Slyke[2] method for the determination of amino acid nitrogen.

It has been known[3] for some time that the method of determining ammonia by distilling with magnesium oxid may give high results, and several attempts have been made to eliminate this source of error. The Folin method seems to be the best one of these, and has given good results when used by several workers.[4]

In applying the Folin method to cheese investigations it became necessary to determine what set of conditions would give the most accurate results and at the same time allow the ammonia-free extract to be used for the Van Slyke method.

The apparatus used for the ammonia determination was arranged in the manner described by Folin in his original article. Air was drawn through the apparatus by means of suction pumps, one pump for each determination. The water pressure was 42 pounds.

The cheese solutions were made by extracting 25 grams of cheese (which had been ground with sand) with 150 cc portions of water at 55° C. until nearly 1,000 cc had been obtained. The volume was then made up to 1,000 cc and filtered.

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[1] Zts. physiol. Chem., 1902–3, **37**: 161.

[2] Ber. d. chem. Gesell., 1910, **43**: 3170.

[3] Hart, Zts. physiol. Chem., 1901, **33**: 347.

[4] Folin, Zts. physiol. Chem., 1902–3, **37**: 161; Kober, J. Amer. Chem. Soc., 1908, **30**: 1131; Gill and Grindley, J. Amer. Chem Soc., 1909, **31**: 1249; Pennington and Greenlee, J. Amer. Chem. Soc., 1910, **32**: 561; Gebellen, Brymeldsen and Haevardsholn, Chem. Ztg., 1909, **33**: 793; Denis, J. Biol. Chem., 1910, **8**: 427.

For the determination of ammonia 100 cc of this solution, equal to 2.5 grams of cheese, were employed. As this is a much larger volume than is used by Folin a few determinations were run on a solution of ammonium chlorids of known strength in order to obtain some idea as to the amount of alkali and the length of time required to drive over all the ammonia.

It was found that 4 grams of sodium hydroxid or sodium carbonate and four hours of aeration would recover all the ammonia from 100 cc of ammonium chlorid solution containing 0.0280 gram of nitrogen.

In using sodium hydroxid, hydrolysis may take place, increasing the ammonia, or phosphates may be precipitated which contain ammonia and give low results. Hydrolysis may be prevented by the addition of sodium chlorid and the precipitation of the phosphates be prevented by the addition of potassium oxalate.[1]

ANALYTICAL RESULTS.

These points were investigated with the results reported in the following tables. Some determinations were made by distilling with magnesium oxid, and these are also given.

Determination of ammonia in cheeses.

[Time of aeration, 4 hours.]

Sample.	Alkali used.	Nitrogen.
Cheddar:		*Gram.*
1	10 cc 40 per cent sodium hydroxid	0.0029
2	do	.0028
3	10 cc 40 per cent sodium hydroxid+5 grams sodium chlorid	.0032
4	do	.0028
5	4 grams sodium carbonate	.0027
6	do	.0028
	Magnesium oxid distilled:	
	First 150 cc	.0034
	Second 150 cc	.0006
	Third 150 cc	.0004
Camembert:		
1	10 cc 40 per cent sodium hydroxid	.0081
2	do	.0081
3	do	.0078
4	do	.0077
5	10 cc 40 per cent sodium hydroxid+10 grams potassium carbonate	.0076
6	do	.0078
7	4 grams sodium carbonate	.0077
8	do	.0078
9	4 grams sodium carbonate+10 grams potassium carbonate	.0077
10	do	.0079
11	do	.0076
12	do	.0081
13	Magnesium oxid distilled:	
	First 150 cc	.0085
	Second 150 cc	.0005
	Third 150 cc	.0003

The results obtained seem to indicate that the Folin method[2] can be used with great advantage for the determination of ammonia in cheese, and that the kind of alkali employed makes practically no difference. This is of great importance if we are to use the solution after it has been freed from ammonia for the determination of amino nitrogen by the Van Slyke method. Sodium hydroxid is to be preferred in this case, for the solution must be neutralized and evaporated somewhat and then brought up to the original volume (100 cc).

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[1] Folin, J. Biol. Chem., 1910, 8:497; Steel, ibid., 1909, 7:365.

[2] After this paper was written one of the authors learned that Folin has improved his method. He now uses a small volume of sample (about 10 cc) and determines the ammonia caught in the acid by the colorimeter.

It is apparent at once that the Van Slyke method for amino nitrogen is applicable to cheese work. The only question arising is that of the particular value in this kind of work. It seems to the writers that the advantage in using the Van Slyke method lies in the fact that an indication as to the extent of the ripening of a cheese may be thus obtained.

This is shown in the following scheme, which was suggested in part by D. D. Van Slyke, the originator of the method.

SCHEME FOR THE PARTIAL ANALYSIS OF THE WATER EXTRACT OF CHEESE.

Make the water extract as usual, filtering through paper before using.

(a) Determine total nitrogen in the extract.

(b) Determine ammonia in the extract by the Folin method.

(c) Determine amino nitrogen by the Van Slyke method, using a portion of the ammonia-free solution from (b) after it has been brought back to original volume.[1]

(d) Treat a portion of the original extract with an equal volume of concentrated hydrochloric acid and boil under a reflux condenser until completely hydrolized. Evaporate the solution to remove the hydrochloric acid and then make up to 100 cc and determine the ammonia by the Folin method.

(e) Determine the amino nitrogen by the Van Slyke method in a portion of the ammonia-free solution from (d) after it has been brought back to original volume.[1]

An analysis made according to this scheme will give:

(1) Nitrogen as ammonia (b).

(2) Amino nitrogen (c).

(3) Amino nitrogen bound in peptid linking (e—c).

(4) Nonamino nitrogen (a—[d+e]). This comes from the portion of the casein molecule containing arginin, histidin, prolin, oxyprolin, and tryptophan.

The ratio e:c indicates the average number of amino acids in the molecule of the peptids composing the mixture called the peptones and caseoses, i. e., it is a chemical index of the extent to which the protein in the extract is broken down. In a dipeptid the ratio is 2, in a tripeptid the ratio is 3, etc.

An analysis of a camembert cheese made according to this scheme gave the following results:

	Per cent.
Total soluble nitrogen	3.39
Nitrogen as ammonia	.43
Nitrogen as amino nitrogen	.25
Nitrogen as ammonia after hydrolysis	.76
Nitrogen as amino nitrogen after hydrolysis	1.80
Amino nitrogen bound in peptid linking	1.05
Nonamino nitrogen	.83

Ratio of e:c=1.80:0.25=7.2.

RECOMMENDATIONS.

It is recommended—

(1) That the Folin method for the determination of ammonia be further studied.

(2) That the Van Slyke method for the determination of amino acid nitrogen be further studied.

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[1] This is done by adding a slight excess of acetic acid, evaporating to less than 100 cc, transferring to a 100 cc graduated flask and making up to volume.

REPORT OF COMMITTEE C ON RECOMMENDATIONS OF REFEREES.

By A. L. WINTON, *Chairman.*

(Food adulteration.)

SPICES.

It is recommended—

(1) That further study be made of the ether extract of paprika, particularly of the index of refraction, with a view to detecting added foreign oils.

Adopted.

(2) That samples of prepared mustard of known composition be submitted to collaborators for determination of crude fiber by the present official methods.

Adopted.

CONDIMENTS OTHER THAN SPICES.

It is recommended—

(1) That such of the methods given in the report of the associate referee as seem valuable in detecting spoilage be further studied. (See p. 118.)

Adopted.

COCOA AND COCOA PRODUCTS.

It is recommended—

(1) That methods for the determination of milk solids in milk chocolate be further studied.

Adopted.

DAIRY PRODUCTS.

It is recommended—

(1) That Paul's method of extracting fat from dairy products be further studied, and that the fat obtained by this method be studied as to its chemical and physical constants.

Adopted.

NITROGENOUS BODIES (MEAT PROTEINS).

It is recommended—

(1) That in Bulletin 107, Revised, page 108, 7 (a), there be added the following sentence: "If desired, 5 to 7 grams of potassium sulphate may be added in addition to the mercury of the Kjeldahl method."

Approved and referred to the association for final action in 1912.

(2) That the same modification of the Kjeldahl method be recognized as provisional for meat extracts, Bulletin 107, Revised, page 114, 7 (a).

Approved and referred to the association for final action in 1912.

(3) That the referee for next year make a further study of the separation of nitrogenous bodies (meat proteins).

Adopted.

WATER IN FOODS.

It is recommended—

(1) That the referee for next year make a comparison of the vacuum method (Bul. 122, p. 219) with the official methods for the determination of moisture in foods; and that he study other desiccating agents than sulphuric acid.

Adopted.

VINEGAR.

It is recommended—

(1) That the method quoted by the associate referee for glycerin (Ross. Bul. 137, pp. 61–63) be adopted as provisional.

Adopted.

(2) That the provisional method for pentosans (Bul. 107, Rev., p. 54) be applied to vinegar, with the direction for the use of the proper amount of hydrochloric acid to allow for the water in the vinegar.

Adopted.

(3) That the other methods proposed by the referee be continued for future study. (See p. 126.)

Adopted.

CEREALS.

It is recommended that the following action be taken in regard to the establishment of methods for the examination of wheat flour:

(1) That the method for the determination of moisture given in Bulletin 107, Revised, page 38 (1), be made official for wheat flour, and that a further study be made of the efficiency of the vacuum desiccator as compared with the vacuum oven.

Adopted; referred to association for final action in 1912.

(2) That Method B of the associate referee's report for the determination of ash be made a provisional method. (See p. 102.)

Adopted; referred to association for final action in 1912.

(3) That methods for the determination of the acidity of the water extract of flour be further studied with reference to the temperature of the water and time of extraction.

Adopted.

(4) That the method for the determination of the ether extract given in Bulletin 107, Revised, page 39, 5 (b) (1), be made official.

Approved and referred to the association for final action in 1912.

(5) That the method of Bryan, Given, and Straughn for the determination of soluble carbohydrates be given a more extended trial. (See statement of method below.)

Adopted.

(6) That protein be calculated from the nitrogen determined by the Kjeldahl or Gunning method, using the factor 5.70.

Adopted.

(7) That Winton's gasoline color value method be made provisional (Bul. 137, p. 144).

Adopted.

(8) That a study be made of methods for the determination of nitrites in flour.

Adopted.

(9) That experiments be made on the feasibility of determining acidity and nitrites in aliquots of the same water extract of flour.

Adopted.

The following statement of the method of Bryan, Given, and Straughn is submitted as showing the form in which it should be considered for adoption; details and discussion given in Circular 71:

BRYAN, GIVEN, STRAUGHN METHOD FOR THE DETERMINATION OF SOLUBLE CARBOHYDRATES.

(a) *Preparation of solution.*—Place 12 grams of the finely-ground material in a 300 cc graduated flask, with 1 to 3 grams of precipitated calcium carbonate to neutralize the acidity, add 150 cc of 50 per cent alcohol by volume (carefully neutralized), mix thoroughly, and boil on a steam bath for one hour, using a small funnel in the neck of the flask to condense the vapor. Cool, make to volume (300 cc) with 95 per cent alcohol (neutral in reaction), mix thoroughly, allow to settle. Transfer 200 cc to a beaker with a pipette, and evaporate on a steam bath to a volume of from 20 to 30 cc, *but not to dryness*. This should remove all but traces of alcohol. Add 20 cc of water, stir, and transfer the solution to a 100 cc graduated flask, washing the beaker with water into the flask. Add to this enough saturated solution of neutral lead acetate from a burette or pipette to produce complete precipitation, but avoid an excess. Allow to stand 15 minutes and make up to a volume of 100 cc with water, shake well, and filter. At least 75 cc of filtrate should be obtained. Add anhydrous sodium carbonate or potassium or sodium oxalate to precipitate all the lead, allow to stand 15 minutes, and pour onto an ashless filter. Test the filtrate for lead with small quantities of the precipitating agents mentioned above and refilter if necessary. This solution represents the sugars from 8 grams of the original material and is used in the following determination.

(b) *Reducing sugars.*—Use 25 cc of the filtrate together with 25 cc of water as the sugar solution for Munson and Walker's method (Bul. 107, Rev., p. 241), or 25 cc of the solution for Allihn's method (ibid., p. 49). With Allihn's method the amount of dextrose found is multiplied by the factor 1.044 to obtain the equivalent in invert sugar.

(c) *Sucrose.*—Place 50 cc of the filtrate from (a) in a covered 400 cc beaker. In case sodium carbonate was used to throw out the lead, add a small piece of litmus paper and neutralize with acetic acid, then add 5 cc of concentrated hydrochloric acid, and allow to stand overnight for inversion. If potassium or sodium oxalate was used for removing lead it is not necessary to neutralize, but the acid can be used direct. At the expiration of 24 hours neutralize with anhydrous sodium carbonate, wash into a 100 cc flask, and make up to the mark. Filter, if necessary, and use 50 cc for the determination of total sugars as invert by the method of Munson and Walker or 25 cc by the method of Allihn. Subtract the percentage of reducing sugars before inversion calculated as invert sugar from the percentage of total invert sugar after inversion and multiply this product by 0.95 to obtain the percentage of sucrose.

For more exact results it is necessary to determine the volume occupied by the 12 grams of material used in this work and to account for it. A large number of determinations has shown the average volume of 12 grams to be 9 cc. Therefore the correction would be 0.97, and, hence, the percentages of original sugars and sucrose should be multiplied by this factor to obtain the most accurate results.

FLAVORING EXTRACTS.

It is recommended—

(1) That the method of determining vanillin, coumarin, normal lead number, and residual color in one weighed portion, as proposed by Winton, Lott, and Berry, be provisionally adopted, changing the text of the method as adopted at the last meeting (Bul. 137, p. 68) so as to include the detail of precipitation at a standard temperature, 37° to 40° C. (Bul. 137, p. 120), and to make quantitative the provisional method for the determination of color in the filtrate, as given in Bulletin 107, Revised, p. 159, 11 (b). (See p. 144.)

Adopted.

(2) That the preceding method for determining vanillin, coumarin, normal lead number, and residual color in filtrate in one weighed portion of sample be further studied next year for the special purpose of ascertaining the limits of composition of standard vanilla extracts.

Adopted.

(3) That Tolman's method for determining per cent of color insoluble in amyl alcohol (Marsh reagent) be adopted as provisional, and that the text of the method as published in the Proceedings of the Association of Official Agricultural Chemists (Bul. 132, p. 90) be inserted as (c) under "11. Test for coloring matter," in Bulletin 107, Revised, page 159. Twenty-five cubic centimeters of the sample is sufficient for the test. It is further recommended that this method be studied next year for the purpose of determining the range of values for pure vanilla extracts.

Adopted.

(4) That the provisional and other methods for the determination of benzaldehyde in almond extract be further studied, with the view to determining

the reliability of the methods and also the conditions under which aldehyde is oxidized to benzoic acid in commercial extracts, as well as the extent of such oxidation. (Bul. 137, p. 74; Cir. 66, p. 21.)

Adopted.

(5) That Mitchell's modification of the Seeker test for ginger be further studied. (See p. 137.)

Adopted.

(6) That the method for the detection of capsicum in ginger extract, as proposed by Doyle, modifying the La Wall method, be adopted as provisional. The Doyle method is not essentially different from La Wall's. The details of procedure, however, are such as to make the test more positive and are set forth more clearly than in the latter method. (See p. 137.)

Approved and referred to the association for final action in 1912.

(7) That the Street-Morrison method (Bul. 137, p. 76) and other available methods for examining and identifying the components of the total solids of ginger extracts be a subject for study next year. Such a method is necessary for proving adulteration in alcoholic extracts of ginger.

Adopted.

(8) That the subject of the determination of oil of nutmeg in nutmeg extract be studied further and that other methods be tried next year, as both of the methods tried this year proved utterly unreliable.

Adopted.

(9) That for the determination of oil of wintergreen in wintergreen extracts both of the following methods be further studied:

First. Howard's method as described in J. Ind. Eng. Chem., 1911, 3:252, using cold dilute sulphuric acid (1:2) for the floating medium.

Second. Hortvet and West's method of saponifying the oil and weighing as salicylic acid. (J. Ind. Eng. Chem., 1909, 1:90.)

Adopted.

(10) That the Howard method (Bul. 137, p. 76) for the determination of oil of peppermint in alcoholic solutions, which was adopted provisionally last year, be given further study. This method has been modified, in the interests of greater accuracy, by its author, and the new method is found in J. Ind. Eng. Chem., 1911, 3:252.

Fats and Oils.

It is recommended—

(1) That further work be done on the Emery method next year. (U. S. Dept. Agr., Bureau of Animal Industry, Cir. 132.)

Adopted.

(2) That the provisional method for the preparation of samples be made official. (Bul. 107, Rev., p. 129.)

Approved for final action in 1912.

(3) That the referee for next year study the advisability of changing the official method for the determination of specific gravity at 100° to a similar method at 75° C.

Adopted.

(4) That method "(c) Zeiss Butyro Refractometer" (Bul. 107, Rev., p. 132) be made official instead of provisional, as at present.

Approved for final action in 1912.

(5) That the provisional method for the melting point of fatty acids (Bul. 107, Rev., p. 135 (b)) be made to include the fat as well as the fatty acids and to read as follows:

Draw the melted fat or fatty acids into a thin-walled capillary tube, 1 inch or 2 inches long, according to the length of the bulb of the thermometer used. Seal one end of the tube and allow the fatty acids to cool on ice from 12 to

15 hours. Attach to the bulb of a delicate thermometer graduated to 0.2°, immerse in a large test tube of water surrounded by a beaker of water, and heat very slowly. An apparatus similar to that indicated for use in the Wiley method, but smaller, will prove satisfactory. The point at which the substance becomes transparent should be taken as the melting point.

Adopted, final action.

(6) That method "12. Determination of free fatty acids" (Bul. 107, Rev., p. 143) be made official instead of provisional, as at present.

Approved for final action in 1912.

(7) That the Halphen reaction for cottonseed oil (Bul. 107, Rev., p. 144, 17(b)) be made official instead of provisional.

Approved for final action in 1912.

(8) That the Bechi or silver nitrate test for cottonseed oil (Bul. 107, Rev., p. 145, 17(c)) be made official.

Approved for final action in 1912.

(9) That the Baudouin test for sesame oil (Bul. 107, Rev., p. 146, 17(e)) be made official.

Approved for final action in 1912.

(10) That the Villavecchia test for sesame oil (Bul. 107, Rev., p. 146, 17(f)) also be made official.

Approved for final action in 1912.

(11) That any cut appearing in the text of the chapter on fats and oils, Bulletin 107, Revised, be considered merely as an illustration and not as an integral part of the method.

Approved for final action in 1912.

A supplementary report on the separation of nitrogenous bodies (milk and cheese) was made by Mr. Street as chairman of committee A (see page 86).

A NOTE ON THE DETERMINATION OF BENZALDEHYDE IN LIQUEURS, DISTILLED LIQUORS, AND CORDIALS.

By F. G. Smith.[1]

The method used consisted of a simple distillation and a precipitation with phenylhydrazin as proposed by Denner[2] and further improved by Denis and Dunbar.[3] Folin and several other investigators claim that many fruit products show about 0.015 per cent benzoic acid by the quantitative methods now in use. This fact makes the oxidation method for the determination of benzaldehyde unreliable for traces. Work done recently on maraschino liqueurs and cherries shows practically no benzaldehyde in genuine maraschino preparations. This would be expected from the fact that maraschino is prepared from a cherry mash and sometimes the distillate is flavored with broken cherry pits. Several artificial maraschino liqueurs were prepared containing a known amount of commercial benzaldehyde, which was assayed by the Denis-Dunbar method and found to contain 87 per cent of this substance. A fractional distillation of the first maraschino liqueur prepared gave about 98 per cent of the benzaldehyde recovered in the first two 10 cc fractions; the next 20 cc fraction recovered the remainder. The total recovery from the 200 cc distilled was about 90 per cent of the benzaldehyde present. This liqueur stood in contact with the air some time after being prepared, and some of the benzaldehyde was undoubtedly lost.

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[1] Presented by Mr. A. S. Mitchell.

[2] Zts. anal. Chem., 1890, 29: 228.

[3] J. Ind. Eng. Chem., 1909, 1: 256.

Several fractional distillations of an artificial maraschino gave the following amounts of benzaldehyde:

Recovery of benzaldehyde by fractional distillation of three 200 cc samples of artificial maraschino.

Number of distillations.	Fraction.	0.10 gram commercial benzaldehyde present.		0.06 gram commercial benzaldehyde present.
		Sample 1: Distilled in current of carbon dioxid.	Sample 2: Distilled in air.	Sample 3: 50 grams powdered sodium chlorid added; distilled in air.
	Cc.	*Gram.*	*Gram.*	*Gram.*
1	10	0.0600	1.0551	0.0468
2	10	.0184	.0193	.0062
3	20	.0070	.0105	.0012
4	20	.0019	.0016	.0019
5	20	.0005	.0000	
6	20	.0023	.0011	
7	20	.0004	.0008	
8	20	.0000	.0000	
Total amount		.0905	.0884	.0591
Per cent recovered		90.5	88.4	93.5

The high per cent of benzaldehyde recovered in the sodium chlorid distillation is due to a probable decomposition of sucrose resulting in volatile substances precipitable by phenylhydrazin. Distillations of maraschino liqueurs prepared with sucrose showed an increased precipitate in the latter fractions, while those prepared entirely of invert sugar, as in the case of No. 2, show no such increase in the last three fractions.

The conditions essential to a complete precipitation by phenylhydrazin were studied on pure solutions of benzaldehyde and on artificial liqueurs prepared in this laboratory as well as on a number of genuine and imitation maraschinos. In this work the volume from which the benzaldehyde was precipitated was kept as small as possible, as a large volume of water seems to increase the decomposition of phenylhydrazin, caused by the action of the light and air.

Alcohol up to 20 per cent by volume in the solution did not affect the determination, as will be shown by the following table, which also shows that an excess of alcohol can be boiled off without affecting the result. This is true of quite large excesses of the reagent, but the precipitate is more difficult to handle.

Benzaldehyde determinations in the presence of varying amounts of alcohol.

[0.10 gram present.]

Number.	Benzaldehyde found.	Alcohol.	Denis and Dunbar No. 2 reagent.
	Gram.	*Per cent by volume.*	*Cc.*
1	0.0872	5	5
2	.0883	10	5
3	.0882	20	5
4	.0817	50	5
5	.0882	[1] 10	5
6	.0886	[1] 20	5
7	.0876	[2] 10	5
8	.0877	[2] 20	5
9	.0868	4	2
10	.0886	4	5
11	.0882	4	10
12	.0882	4	15

[1] Alcohol boiled out.

[2] Added after boiling out.

The solubility of a pure benzalphenylhydrazone in distilled water was found to be less than 3 parts in 100,000 and is not appreciably increased by a small per cent of alcohol. The 12 determinations on a 0.10-gram sample of the commercial product used gave in each case within 0.5 mg of 0.088 gram of benzaldehyde.

The method used for weighing the precipitated benzalphenylhydrazone was found to give admirable results. It consisted in balancing two 9-cm filter papers, filtering through the two papers superimposed, and counterbalancing the one against the other in weighing the precipitate. The precipitates were dried in a 60° air oven and overnight in a sulphuric acid desiccator. Four determinations were made on the benzaldehyde used, with the following results:

Commercial benzaldehyde.	Benzaldehyde recovered.
0.1	0.0883
.01	.0078
.001	.0009
.0001	slight but distinct precipitate.

One milligram of benzaldehyde was added to 100 cc of distilled water and distilled in a current of carbon dioxid. Practically all of the benzaldehyde was recovered.

The factor for the conversion of benzalphenylhydrazone into benzaldehyde is 0.5408, and for benzal-semicarbazid to benzaldehyde 0.7213.

Although distillation in air seems usually to recover the full amount of the benzaldehyde, a distillation in carbon dioxid always does, and for this reason is to be preferred. It was found helpful to wash the precipitate once in each case with dilute acetic acid.

Several samples of imported maraschino cherries, probably genuine, and two imported maraschino liqueurs were found to contain the following amounts of benzaldehyde:

	Grams per 100 cc.
Maraschino liqueur	[1] 0.0018
Do	.0000
Maraschino cherries, juice	.0001
Do	.0000
Do	.0003
Do	.0000
Imitation products:	
Maraschino liqueur	.0150
Maraschino cherries, juice	.0065
Do	.0177
Do	.0062
Do	.0000
Do	.0160
Do	.0120
Do	.0035
Do	.0100
Do	.0232
Do	.0333

The benzalphenylhydrazone in each case is identified by the melting point and by the Melzer test (see page 195), which gives positive results with 1 mg of benzaldehyde or directly with 2 or 3 mg of the benzalphenylhydrazone. In all cases the precipitate was found to be quite pure benzalphenylhydrazone if appreciable in amount.

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[1] The melting point of this precipitate was very low, indicating that it was not benzalphenylhydrazone, but a sugar decomposition product.

The precipitation of benzaldehyde by semicarbazid hydrochlorid as benzal semicarbazone was found to give very concordant results and to agree very closely with the phenylhydrazone method.

Benzaldehyde recovered from 0.10 gram commerical product.

Precipitated in 20 per cent alcohol	0.0888
Precipitated in 50 per cent alcohol	.0436
0.01 gram commercial	.0078
0.001 gram commercial. No precipitate.	
Alcohol boiled out	.0888
Ammonia added	.0916
Acetic acid added	.0892

When using the semicarbazid method the precipitate should be allowed to stand overnight, as it does not form as readily as the phenylhydrazone. The semicarbazone formed is not contaminated with decomposition products as in the case of the hydrazone and it is much easier to handle. The phenylhydrazin, however, is much more delicate in detecting traces, as the semicarbazid hydrochlorid gives no precipitate with amounts up to 2 or 3 mg. The phenylsemicarbazid hydrochlorid was dissolved in water to make a 1 per cent solution. This reagent is fairly stable while the phenylhydrazin must be frequently redistilled and always prepared absolutely fresh.

Melzer method for the detection of benzaldehyde and phenol.[1]—Add to 1 cc of phenol solution, as an aqueous distillate, 2 cc concentrated sulphuric acid, and 1 to 2 drops of benzaldehyde and boil. To test for benzaldehyde, add phenol. The initial yellowish brown color becomes dark red and a red resinous substance separates out on dilution. Let cool and add 10 cc of water and 20 per cent potassium hydroxid until distinctly alkaline. Phenol, in the presence of benzaldehyde, gives a blue violet color. The resultant dye can be extracted from the acidified solution with ether. The method is sensitive to 0.005 gram of phenol.

NOTE ON A TENTATIVE METHOD FOR THE DETERMINATION OF ESSENTIAL OIL IN ALCOHOLIC SOLUTIONS.

By R. S. Hiltner.

Owing to the apparent failure of existing methods for the determination of oil of peppermint and of nutmeg in the so-called extracts the associate referee has attempted to devise a new method that will give more satisfactory results. He offers the following only tentatively as an outline of a process that seems promising:

Prepare a quantity of hydrocarbon oil (boiling at 175° to 200° C.), by fractional distillation of commercial kerosene, pipette 10 cc of the extract sample into a Babcock milk bottle, add 1 cc (accurately measured) of hydrocarbon oil, 2 drops of concentrated hydrochloric acid, and 25 cc of hot water; shake vigorously, warm on the steam bath to about 80°, whirl in a centrifuge, add water so as to bring the floating oil into the neck of the flask, and again whirl. Determine the refractive index of the separated oil and of the hydrocarbon oil used at 20° C. To ascertain the quality of the oil present in the sample it may be necessary to float on water the essential oil in another portion, and determine its refractive index, following in a general way the procedure for determining lemon oil as given in Bulletin 107, Revised, page 156, paragraph 4 (a). It appears possible to calculate the percentage of essential oil from these data.

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[1] Zts. anal. Chem., 1898, 37: 345.

Mr. Winton called attention to the fact that it was very necessary that the methods used by the Government chemists in work done under the food law in determining nitrites in flour should have the approval of the association and be given the standing of official methods. To this end it was moved that permission be given to insert these methods in the Proceedings of 1911 at the close of the report of the referee on cereals, in order that they might be before the association with a view to their adoption at the next meeting.

The motion was carried. (See p. 113 for statement of methods.)

THIRD DAY.

WEDNESDAY—MORNING SESSION.

The Secretary of Agriculture addressed the association at the opening of the session, calling attention especially to the varied activities of the Department of Agriculture, the many problems waiting solution, and the fundamental part played in nearly all of the work by the chemists. Good and cheap food, reliable fertilizers and insecticides, good roads, and cold storage in its bearing on both supply and quality were among the topics discussed. The Secretary in closing expressed his desire to facilitate the work of the association in every way possible, with the cooperation of Dr. Wiley as secretary of the association.

Mr. Withers, as chairman of the committee on a journal of agricultural research, reported that Congress had been asked to appropriate $20,000 for the publication, in the form of a journal, of the original and technical work of the experiment stations. The resolution adopted by the association at its last meeting indorsing this recommendation was duly transmitted by the committee as requested. The item in the appropriation bill was thrown out by the House upon a point of order, and later inserted by the Senate, but was finally stricken out in conference.

As it appeared to the committee impracticable to take further action at this time they requested to be discharged.

The report was accepted and the committee discharged as recommended.

REPORT ON FEEDS AND FEEDING STUFFS.

By G. M. MacNider, *Referee.*

Acidity of Feeds.

The work on the determination of the acidity of feeds has not been pushed this year, as the determination of acidity is of little consequence in estimating the value of a feed except in the case of gluten feeds and feeds which may spoil by fermentation, etc. In such cases the estimation of the water-soluble acidity gives the desired information in regard to the feed. For the determination of the total soluble acidity the method proposed in the last report affords a rapid and accurate means of estimating the acidity of a feed. The method is as follows:

Weigh 10 grams of the sample into a shaking bottle, add 200 cc distilled water, and shake for 15 minutes. Filter the extract through a folded filter and take an aliquot of 20 cc (equal to 1 gram of sample) for the titration. Dilute with 50 cc distilled water and titrate with standard decinormal sodium hydroxid solution, using phenolphthalein as indicator.

The recommendation of the committee "that the subject of the acidity of commercial feeds be studied with special reference to eliminating the error due to the proteins" has not been taken up. To determine satisfactorily the effect of the acidity of the proteins would require a thorough investigation into the reactions of the different proteins occurring in the various feeds, and it was not thought advisable to take up such a piece of research with this problem.

As gluten feeds usually show a higher acidity than other feeds and are among the few which are treated chemically during the process of manufacture, the nature of their acidity has received considerable attention by a number of stations. The references to this literature are given in the last report. Jordan[1] has recently published the results of investigations on the "acidity of gluten feeds." In this work the nature of the acidity of the feeds and also of the steep water, which is added to the feed during the process of manufacture, was studied. The author concludes that the acidity of gluten feeds is caused by the addition of steep water, a by-product obtained in the manufacture of corn products. The steep water contains two constituents which give it an acid reaction, namely, amino acids and phosphorus compounds. The steep water contains traces of mineral acids. They are present in such small quantities that they need not be considered as imparting any deleterious properties to the feed when steep water is added to it. This work bears out the statements which have previously been made in regard to the acidity of gluten feed; i. e., that the acidity is not caused by the presence of mineral acids; and it is, therefore, misleading to report the acidity of a feed in terms of a mineral acid.

Comparison of the Official (Ethyl Ether) Method for Determining Fat with the Petroleum Ether Method.

The petroleum ether method for determining fat in cottonseed products was compared with the official method, as recommended, to ascertain the difference in the results by the two methods. The petroleum ether method, which has been adopted by the Cottonseed Crushers Association for determining oil in cottonseed products, is as follows: Extract 2 to 5 grams of the meal, without previous drying, for three hours in a Soxhlet apparatus with petroleum ether boiling under 65° C. Then evaporate off the ether, weigh the residue, and report as oil.

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[1] New York (Geneva) Agr. Exper. Sta., Tech. Bul. No. 16.

ANALYTICAL DATA.

The following comparative work has been done:

Cooperative work on fat in cottonseed meal by two methods.

Number and analyst.	Official ethyl ether method.	Petroleum ether method.
F. D. Fuller, Indiana:[1]	*Per cent.*	*Per cent.*
08	8.31	8.02
09	9.18	9.13
010	6.55	5.85
011	8.38	8.21
012	8.92	8.54
013	7.84	7.81
014	8.24	7.87
015	7.83	7.25
016	8.60	8.30
017	7.59	7.26
018	7.14	6.79
019	8.31	8.34
020	12.52	12.53
021	7.71	7.79
022	8.32	8.34
023	7.78	7.25
024	9.02	8.46
025	8.08	7.64
026	4.57	4.53
G. M. MacNider, North Carolina:		
4290	7.72	7.30
4291	9.54	9.17
4292	8.10	7.73
G. M. MacNider, North Carolina—Continued.	*Per cent.*	*Per cent.*
4294	7.47	7.12
4296	8.16	7.93
4297	7.35	7.20
G. L. Bidwell and C. E. Goodrich, Washington, D. C.:[1]		
A	7.39	7.51
B	8.12	7.90
C	5.63	5.62
D	4.71	4.80
E[2]	10.69	10.22
J. B. Herron, Texas:		
1	7.58	7.18
2	8.73	8.60
3	6.78	6.54
4	8.18	8.03
5	6.10	5.80
6	8.56	8.26
7	6.69	6.86
8	7.75	7.60
9	7.85	7.91
10	8.63	8.63

[1] Results are averages of two or more determinations.

[2] Cold-press meal.

The average difference in the results is 0.29 per cent in favor of the ethyl ether; the greatest difference is 0.70 per cent, the smallest 0.01 per cent. With eight determinations the petroleum ether gives slightly higher results than the ethyl ether.

COMMENTS BY ANALYSTS.

F. D. Fuller: The results obtained, with few exceptions, show what might be expected, namely, that petroleum ether boiling under 65° C. removes less fat than does ethyl ether. Inasmuch as ethyl ether dissolves chlorophyl, resins, gums, alkaloids, and similar bodies in respect to which petroleum ether is almost inert it appears that the extract obtained by using petroleum ether more nearly represents the amount of true fat than is the case when ethyl ether is used as the solvent, although lack of time prevented a comparative study of the fat constants.

The advantages of using petroleum ether are obvious—first, the extraction is made without previous removal of moisture; second, the extraction is completed in three hours; third, the petroleum ether can be obtained for approximately 30 cents a gallon, an item which naturally appeals to the commercial chemist; and fourth, the extract undoubtedly more nearly represents the pure fat content of the substance under examination.

The chief disadvantages in using petroleum ether are that at least two hours are required to obtain an extract whose weight is constant, and that petroleum ether does not remove certain substances soluble in ethyl ether which heretofore have been counted as fat and in many cases considered as such in experiments in animal nutrition.

G. M. MacNider: The advantages of the petroleum ether method for the commercial chemist are very great—first, the cost of the petroleum ether is very much less than ethyl ether; second, the sample does not have to be dried before extraction, and the time required for extraction is three hours against sixteen hours by the official record.

G. L. Bidwell: I do not like to see the petroleum-ether method made official and used in all cases on cottonseed meals. In some samples it runs higher, and in others lower than the official method. I believe that it does have value as a control method in the mills, but think the variations from the official method are too great.

From these data it is seen that the average difference in the results by the two methods is 0.29 per cent in favor of the ethyl ether. From other work which has been done this average seems to represent the difference which may usually be expected between the two methods. It is known that the extract by petroleum ether represents more nearly the actual fat than the ethyl ether extract. Petroleum ether is very nearly inert in regard to chlorophyl and other vegetable compounds which are readily soluble in ethyl ether. The saving to the commercial chemist when the petroleum-ether method is used in both the cost of the solvent and the time required to make the analysis is very large, and it therefore seems advisable for the association to recognize this method.

COMPARISON OF THE PROPOSED MODIFICATION OF THE METHOD FOR THE DETERMINATION OF CRUDE FIBER WITH THE OFFICIAL METHOD.

In compliance with the recommendation of the committee, the proposed modification of the official method for the determination of crude fiber was studied. The method is as follows:

Place 2 grams of the sample in a wide-mouth Erlenmeyer flask of liter size, inserting a small air condenser in the mouth of the flask to prevent concentration, due to the loss of steam. To the sample add 200 cc of a 1.25 per cent solution of boiling sulphuric acid, as in the official method. Heat to boiling, and after boiling for thirty minutes treat as follows: Neutralize with a 10 per cent solution of sodium hydroxid, using a few drops of phenolphthalein as indicator, approximately 25 cc of sodium hydroxid are required. Add at once 200 cc of a 2.656 per cent solution of boiling sodium hydroxid and continue the digestion at the boiling point for thirty minutes longer, in the same manner as in the treatment with acid. Then filter the alkaline solution containing the fiber residue through a linen cloth rapidly and wash repeatedly with boiling water. Transfer the fiber residue to a tared platinum Gooch crucible and wash with alcohol and ether. Dry at 100° C. and weigh. Ignite the dried residue and again weigh, the loss in weight giving the weight of fiber.

ANALYTICAL RESULTS.

The following comparative work has been done:

Comparison of the proposed modified method with the official method for determining crude fiber.

Number of sample and analyst.	Sample.	Official method.	Proposed method.
N. H. Ulman, Pennsylvania: [1] [2]			
5272	Blatchford's calf meal	6.61	8.02
5274	Blue ribbon dairy feed	10.48	12.80
5278	Cottonseed feed	28.35	31.22
5343	Alfalfa meal	28.35	30.00
5351	Cottonseed meal	9.52	13.78
5378	Gluten feed	6.55	8.10
E. C. Gildroy, Pennsylvania: [1] [2]			
5272	Blatchford's calf meal	6.61	6.76
5274	Blue ribbon dairy feed	10.48	11.48
5278	Cottonseed feed	28.35	31.32
5343	Alfalfa meal	28.35	28.04
5351	Cottonseed meal	9.52	11.07
5378	Gluten feed	6.55	7.85
H. Hill, North Carolina:			
3951	Cottonseed feed	22.15	22.85
3976	Corn chops	8.10	8.59
3984	Wheat bran	9.18	9.32
3902	Scocotes	9.59	9.88
3933	Middlings	1.37	1.45
3940	Shipstuff	6.35	6.26
G. L. Bidwell and C. E. Goodrich, Washington, D. C.: [1]			
C	Cottonseed feed	22.33	21.39
D	do	20.87	20.57
E	Cold press meal	24.18	24.50
G	Corn product	8.23	8.56
H	Wheat bran	9.67	11.25
I	do	11.15	11.46
J	Hen feed	3.42	3.75
K	Gluten feed	7.86	8.53
L	Feed meal	6.13	6.64

[1] Results are averages of two or more determinations.

[2] Reported by James W. Kellogg.

COMMENTS BY ANALYSTS.

James W. Kellogg: It takes from two to seven hours to filter the residue through the Gooch crucibles after the final washing of the residues has been accomplished. It took about two hours to filter the samples through the linen cloths, which possibly might have been due to the fact that they became very mucilaginous after boiling in the presence of both the acid and the alkali. This condition was undoubtedly the cause of the slow filtrations through the Gooch crucibles. The determination for crude fiber on these samples by the official method occupies less than two hours for the boiling and filtration through the gooch. The proposed method is very unsatisfactory and an unreliable method to use.

H. Hill: The large amount of solution present with the alkali digestion is very inconvenient, causing slow boiling and excessive bumping. There is uncertainty about reaching the exact neutral point when neutralizing cottonseed feeds with the 2.656 per cent sodium hydroxid solution, due to the red color of the acid solution. Filtration through the linen was slow and through the gooch very slow. More time was required to make 6 determinations by the new method than is required to make 12 determinations by the official method. The method is expensive, more sodium hydroxid is required, and considerable alcohol and ether are wasted with each determination. The new method is more inconvenient, consumes more time, and gives higher results than the official method.

G. L. Bidwell: The proposed crude fiber method is not satisfactory. There is no great gain in using it, and the results are not strictly comparable with those given by the official method.

These results cover quite a wide range of feeds and would appear to constitute a fair trial. As will be seen from the table, the results by the proposed method are, in all but four cases, higher than the results by the official method. The greatest difference between the results by the two methods is 4.26 per cent, the smallest 0.08 per cent. In many of the determinations the results by the proposed method are higher than those by the official method by 1 per cent and more.

The results obtained by the two Pennsylvania chemists, working separately on the same samples, show a wide variation, while each chemist was able to obtain satisfactory checks on his own work, and, as these chemists found no difficulty in checking each other's work by the official method, this indicates that it is difficult to obtain satisfactory results by the proposed procedure. The plan of treating the samples with alcohol and ether after the digestions are made is not a good one, as with feeds high in fat the fat will unquestionably reduce the solvent action of the acid and alkali on the sample. It is seen, therefore, that the proposed method gives higher results than the present official method, requires more time, and is more difficult to operate.

RECOMMENDATIONS.

It is recommended—

(1) That the method suggested for determining the acidity of feeds be provisionally adopted. (See p. 198.)

Also that in reporting the acidity of feeds the results be stated in terms of cubic centimeters of the sodium hydroxid used or its equivalent in grams of sodium hydroxid.

(2) That the association recognize the petroleum ether method for determining fat in cottonseed products.

(3) That the proposed modification of the official method for the determination of crude fiber be no further considered.

(4) (On account of lack of time neither the referee nor the associate referee has been able to take up the study of the protein factor and therefore the following recommendation should be carried over to another year.)

That the referee make a study of the literature on the amount of protein in the various vegetable products used for feed, and report to the association at the 1912 meeting on the advisability of adopting more accurate factors for converting nitrogen into protein than the present factor of 6.25.

REPORT ON SUGAR AND MOLASSES.

By WILLIAM E. CROSS, *Referee.*

Owing to the late date of my taking up the work as referee (May) it was thought advisable to confine the investigations almost entirely to the recommendations of last year. Two samples only were sent out, the previous year's experience having shown the difficulty of securing cooperation when too much work was given. The samples consisted of (1) No. 16, a raw Cuban sugar, and (2) No. 18, a centrifugal molasses.

The following instructions accompanied the samples sent to the various cooperators:

INSTRUCTIONS FOR COOPERATIVE WORK.

MOISTURE—(MOLASSES).

Sample A.

Method 1. Determine moisture in 2 grams of sample by heating 10 hours at 100° C. with sand, according to method 3, page 65, Bulletin 107, Revised.

Method 1a. Determine moisture by heating 2 grams of sample for 10 hours at 100° C. without sand.

Method 2. Determine moisture in 2 grams of sample by heating on 6 grams of sand in vacuum at 70° C. Note number of hours required to obtain constant weight.

Method 3. Determine moisture in 2 grams of sample by following method: Weigh out into dish containing 50 grams of sand (free from iron). Warm in an oven, then add a little distilled water and mix thoroughly. Dry at 70° C. for 2 hours in a steam oven and thereafter, till weight is constant, in vacuo. Weight judged constant when 2 hours' further warming gives less than 0.1 per cent decrease.[1]

Method 3a. Repeat 3, but heat in vacuum at 100–105° C., instead of 70° C.[1]

Method 4. Determine moisture by Brix spindle. (See areometric method (1), p. 65, Bul. 107, Rev.)

Method 5. Refractometer. Make refractive index reading and convert to percentage moisture by means of Geerligs's tables:

(a) In a concentrated form.
(b) Half diluted with water.
(c) Half diluted with a saturated solution of sugar, using formula—

$$X=\frac{(A+B)\ C-BD}{A}$$

A=weight of material taken and mixed with B.
B=weight of sugar solution.
C=per cent dry substance of above mixture, obtained from refractive index.
D=per cent dry substance of pure sugar solution obtained from refractive reading.

MOISTURE—(SUGAR).

Sample B.

1. Determine moisture by official method, page 64, Bulletin 107, Revised.
2. Determine moisture by refractometric determination of solution of sugar in own weight of water.

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[1] Modification of German official method.

POLARIZATION (SUGAR AND MOLASSES).

1. Weigh out normal weight, clarify (lead subacetate solution), make up to 100 cc (or more if necessary for accurate polarization), and polarize (according to Method c, Bul. 107, Rev., p. 40), single and Clerget.
2. Weigh out normal weight, make up to volume required, clarify with Horne's dry lead subacetate (J. Amer. Chem. Soc., 1904, 26:186) and polarize, single and Clerget.
3. (Molasses only.) Weigh out normal weight, add lead subacetate solution, make up to 200 cc, filter; to 50 cc of filtrate add 1 cc of 30 per cent acetic acid and 2 cc of alumina cream, make up to 55, filter, and measure, single polarization.

3a. Repeat 3, using Horne's dry lead instead of subacetate solution. (Record all dilutions and polarization readings, single and invert, and temperatures.)

It is important that work on samples should be commenced as soon as possible after their receipt on account of fermentation changes which are liable to occur. * * *

RESULTS OBTAINED.

MOISTURE CONTENT OF MOLASSES.

The main object of the work prescribed on moisture methods was to continue the study of the application of the refractometric method to those molasses, etc., which are too dark to be read directly. It was also intended to secure additional data on the comparison between the provisionally prescribed method of drying at atmospheric pressure with the vacuum drying prescribed for substances containing much levulose, etc., and with the drying method officially prescribed in Germany. Owing probably to the awkwardness of the vacuum determinations, however, very little cooperation along these lines was secured.

The following table shows the results obtained on moisture methods:

TABLE 1.—*Comparative percentage results on the determination of moisture in molasses.*

Name.	Heating 10 hours at 100° C. with sand.	Heating 10 hours at 100° C. without sand.	Vacuum at 70° C.	Hours.	Vacuum at 70° C., 50 grams sand.	Hours.	Brix.	Refractometer.		
								Undiluted.	Diluted with water.	Diluted with sugar solution.
A. Given, Washington, D. C.	21.37	21.26	21.10	25	22.3	26	16.76	20.25	19.04	18.10
W. E. Cross, New Orleans, La.	20.42	20.45	......	......	......	......	17.20	20.73	19.24	20.23
S. F. Sherwood, Washington, D. C.	21.96	21.25	20.15	26	20.70	26	16.50	20.35	19.72	20.64
W. D. Horne, New York, N. Y.	22.15	20.81	19.43	......	......	......	18.36	......	......	......
W. G. Taggart, New Orleans, La.	21.21	20.32	......	......	......	......	17.24	20.50	......	......
H. Z. E. Perkins, New Orleans, La.	21.55	20.18	......	......	......	......	......	......	......	......
R. S. Hiltner, Denver, Colo.	21.96	21.40	20.80	20	20.70	20	17.80	20.70	20.10	21.00
G. H. Hardin, New York, N. Y.	20.90	......	......	......	......	......	18.50	20.60	20.10	20.87
H. M. Shilstone, New Orleans, La.	......	......	......	......	......	......	......	21.10	20.37	20.70
Average.	21.44	20.81	20.37	......	21.12	......	17.48	20.60	19.76	20.25

Taking first of all the results on the refractometric method, it will be seen that the work entirely confirms the results obtained in previous years, proving that the error brought about by diluting the dark-colored solution is reduced to a minimum by using a sugar solution as a diluent. To investigate this question further, a number of other samples were tested by a small number of cooperators, working on the same instrument, thereby making conditions as nearly as possible uniform throughout the work. The results of this are shown in Table 2.

TABLE 2.—*Moisture determinations with the refractometer.*

Description of sample.	Name of analyst.	Undiluted.	Diluted with water.	Diluted with sugar solution.
		Per cent.	*Per cent.*	*Per cent.*
Sample B: Second molasses	W. E. Cross	20.75	18.76	20.50
	H. M. Shilstone	21.05	19.70	20.10
	W. G. Taggart	21.00	20.50	20.87
Average		20.93	19.65	20.49
Sample C: First molasses	W. E. Cross	20.93	19.70	21.09
	W. G. Taggart	21.40	21.10	21.57
	H. M. Shilstone	21.10	20.37	20.70
Average		21.14	20.39	21.12
Sample D: Sirup	W. E. Cross	35.65	35.20	35.57
	H. M. Shilstone	36.40	36.32	36.12
Average		36.02	35.76	35.85
General average		24.78	23.95	24.57

These results are in entire agreement with those obtained last year, as the following table shows:

TABLE 3.—*Comparison of moisture data obtained in 1910 and 1911.*

Data.	Undiluted.	Diluted with water.	Diluted sugar solution.
1910 average (4 samples)	24.71	23.82	24.09
1911 average from Table 1	20.60	19.76	20.25
1911 average from Table 2	24.78	23.95	24.57

In all of these determinations it is shown that by using a molasses diluted with sugar solution the results obtained more nearly approximate the true refractometric reading—i. e., on the undiluted solution—than when water is used as a diluent. As this is also, of course, in full accord with theoretical reasoning, it would appear desirable to modify the "provisionally" prescribed method by stating that when dilution is necessary a sugar solution should be used.

Another point brought out by the figures of this year, as well as by those of previous years, is the fact that the official aerometric method with the Brix spindle gives results which are entirely removed from what the drying and refractometric methods indicate to be the true values. The following table shows this very clearly.

TABLE 4.—*Total solids by Brix compared with results by other methods.*

Substance.	Drying at 100° C. 10 hours (average).	Drying in vacuum.	Refractometer.	Average.	Brix.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	
A: First centrifugal molasses (1911)	78.88	79.63	79.40	79.30	82.52
B: High-grade sirup (1910)	73.54	72.82	73.03	73.13	74.05
C: High-grade open-kettle molasses (1910)	74.53	73.71	74.51	74.25	75.21
D: Centrifugal molasses (1910)	74.11	73.83	74.70	74.21	76.20
E: Mixture of blackstrap and glucose	77.59	77.81	78.92	78.11	83.33

We are taught by all scientific opinion and practice to consider the results obtained by the drying method (and especially the drying vacuum method) as the closest approximation to the correct value, and the refractometric results also support this view. It, therefore, seems that the Brix spindle method gives results which are very far from correct, and there is no apparent reason why this method should, in face of this evidence, continue as an official method. I believe I am right in saying that samples C and D of Table 4 would afford ground for prosecution under the pure food law, although the Brix value is higher than 75 per cent in each case.[1]

Another question of importance is the necessity or otherwise of the use of sand in the drying method. It is the custom in very many laboratories to determine moisture by the simple heating for 10 hours without sand; in fact, I believe I am right in stating that the method of drying with sand has only entered into routine work to a very small extent. In the following table the average results obtained by heating 10 hours with and without sand are compared.

TABLE 5.—*Moisture determinations with and without sand.*

Sample.	With sand.	Without sand.
	Per cent.	*Per cent.*
Molasses, 1911	21.44	20.81
Molasses, 1910	24.80	24.38
Sirup, 1910	26.60	26.33
Average	24.28	23.85

The results by the drying method without sand thus approximate reasonably those by the vacuum method and the method of drying with sand. In view of the comparative awkwardness of the sand-drying method, and of the extensive use of the method of drying without sand, it might seem desirable to give some sort of recognition to the latter method.

MOISTURE CONTENT OF SUGARS.

An attempt was made to compare the ordinary method of determining the moisture content of sugar by drying with a refractometric method, in which the raw sugar was dissolved in its own weight of water and the refractive index of the solution taken. The results of the work, however, were so various that no conclusions whatever could be drawn therefrom, so that the question must be left over to another year.

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[1] I am aware of the rider on page 65, Bulletin 107, Revised, to the effect that the aerometric method does not apply to "low-grade products," but all of the samples in Table 4, except E, are technically "high grade" products.

TABLE 6.—*Moisture in raw sugars.*

Name.	By official method.	By refractometer.
	Per cent.	*Per cent.*
W G. Taggart, New Orleans, La.	1.09	0.48
S. F. Sherwood, Washington, D. C.	1.16	.86
R. S. Hiltner, Denver, Colo.	1.00	.30
G. H. Hardin, New York, N. Y.	1.05	1.10

POLARIZATION.

The work of last year showed that the use of Horne's dry lead subacetate as a clarifying agent gave results almost identical with those obtained with lead subacetate solution. This question was further investigated this year and the results are given in Table 7.

TABLE 7.—*Polarization results using normal quantity of clarifying agents.*

Names.	Lead subacetate.				Horne's dry lead.				Molasses.	
	Molasses.		Sugar.		Molasses.		Sugar.		Method 3.	Method 3a.
	Single.	Clerget.	Single.	Clerget.	Single.	Clerget.	Single.	Clerget.		
A. Given, Washington, D. C.	31.00	38.66	96.50	96.51	30.50	38.74	96.35	97.00	31.00	31.00
S. F. Sherwood, Washington, D. C.	31.20	39.19	96.60	96.38	....	....	96.50	96.03	30.90	30.80
W. D. Horne, New York, N. Y.	30.80	38.92	96.38	96.36	30.80	38.42	96.12	96.16	30.58	....
H. Z. E. Perkins, New Orleans, La.	....	....	95.78	95.55	....	....	95.68	95.37	32.05	31.68
B. J. W. Pearce, New Orleans, La.	31.60	38.53	95.90	96.35	31.64	38.70	95.88	96.06	31.19	31.70
W. H. Hoffman, jr., New Orleans, La.	31.20	38.03	96.00	96.50	31.20	38.80	96.20	97.31	31.24	31.24
W. E. Cross, New Orleans, La.	32.20	39.35	96.40	97.73	32.32	39.33	96.25	97.57	31.57	31.68
W. G. Taggart, New Orleans, La.	32.00	39.65	96.35	97.88	32.00	39.38	96.35	97.79	31.80	31.85
R. S. Hiltner, Denver, Colo.	29.70	37.90	95.57	97.30	29.90	37.50	95.57	96.60	30.20	30.36
Average	31.21	38.78	96.16	96.73	31.19	38.69	96.10	96.65	31.17	31.29

SUMMARY OF AVERAGE RESULTS.

Methods.	Molasses.		Sugar.	
	Single.	Clerget.	Single.	Clerget.
Dry lead	31.19	38.69	96.10	96.65
Lead subacetate solution	31.21	38.78	96.16	96.73
Difference	-.02	-.09	-.06	-.08

These results confirm those of previous years, showing that, properly employed, the Horne's dry-lead method gives results practically identical with those of the standard method. In previous years the proposal has been made that the dry-lead method should be made the sole official one, as it has the advantage of eliminating the error due to the lead precipitate. While the evidence would seem to show that this step is justified, the writer feels that as it is against or, shall we say in advance of, almost all home and foreign practice, very careful consideration should be given to the matter before the recommendation is adopted.

The work on the polarization of molasses by methods 3 and 3a was intended to investigate the effect of removing the error due to the influence of the alkaline lead solution on the specific rotation of the levulose present. Unless there was a considerable excess of lead there would be no levulose removed in the precipitate; and as the filtrate was restored to slight acidity with acetic acid, the levulose would have its normal value. However, as is seen from Table 7, almost identical results were obtained with this as with the ordinary method.

On account of pressure of other matters, it was found impossible to carry out any work on recommendations 2 and 4 of last year.

RECOMMENDATIONS.

It is recommended—

(1) That Herles's solution and neutral lead acetate be adopted provisionally as clarifying agents.[1]

(2) That the provisionally adopted method for the refractometric determination of moisture in molasses, etc., should include instructions to dilute the molasses with a pure sucrose solution, instead of water, when the molasses is too dark to allow of direct reading.

(3) That, since the official areometric method of determining "total solids" gives with all molasses, sirups, etc., results which are admittedly too high, the advisability of retaining this as an official method should be considered.

(4) That consideration should be given to the advisability of giving some recognition to the method of drying molasses without sand, a method which is widely used in actual work and which gives results which approximate fairly closely those of the provisionally adopted drying method.

(5) That the advisability of adopting Horne's dry-lead clarification as the sole official method be discussed.

(6) That further work should be done on the determination of moisture in sugars by means of the refractometer, on the influence of the composition of the basic lead acetate (as clarifying agent) on the polarization of sugar solutions, and on the factors used in the formula for determining commercial glucose.

TEMPERATURE CORRECTIONS IN RAW SUGAR POLARIZATIONS.

By W. D. HORNE.

In polarizing raw sugars at temperatures other than the standard 20° C. errors are introduced that are of serious moment. Much has been written on the subject, and temperature corrections have been worked out, but unfortunately they have not been generally adopted. One of the best methods of effecting the necessary corrections is that described by C. A. Browne at the Seventh International Congress of Applied Chemistry, to be described later; and the present paper is to present results on many hundreds of analyses, which entirely corroborate the reliability of this method of correction.

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[1] This recommendation was sent in last year, but arrived too late to be considered by the committee. The present referee regards the results of last (and previous) year's work on these clarifying agents as entirely justifying this recommendation.

In the spring of 1909, when the New York Sugar Trade Laboratory was planned, I made some preliminary experiments on temperature corrections in my own laboratory to ascertain the relative results of polarizing at 20° C. and at higher temperatures, and later I have made continued close comparisons between the polarizations of the Sugar Trade Laboratory at 20° C. and the polarization of the same sugars in other laboratories at ordinary room temperatures.

In the early experiments it was necessary, for polarizing at 20° C.—

(1) That the solution of the normal weight of sugar should be made up to 100 cc at 20° C.

(2) That it should be polarized at 20° C.

(3) That the polariscope should be at 20° C.

In order to accomplish these ends I cooled the solution of sugar, in a flask containing a thermometer, in ice water to 20° C., filling to the mark with a few drops of water at about this same temperature. The solution was then shaken and filtered at room temperature, which necessarily warmed it up a little, as the laboratory was usually above 20° C. This filtrate was put into a tubulated 200 mm observation tube, containing a centrally located thermometer, and immersed in ice water until the temperature fell a little below 20° C. (about 18°), dried with a towel, two protecting caps containing granular soda lime to prevent condensation of atmospheric moisture on the cold end glasses were slipped on, and the whole was put into the polariscope. These caps fit tightly over the ends of the observation tube, each having a glass-windowed end and an annular space inside filled with soda lime, which was found to be much better for this purpose than calcium chlorid or caustic alkali.

The polariscope was kept at 20° C. by inclosing the working parts, including both polarizing and analyzing nicols, in a galvanized-iron box covered with a ⅝-inch layer of cork board and containing two ¼-inch copper tubes running along each side of the inside of the box, through which ice-cold water was circulated.

It was found advisable to pass water at 1° C. through this tube at the rate of 100 cc per minute, which cooled the polariscope to 20° C., as shown by a thermometer passing through the box and covering near the analyzer and compensating quartz wedges. The water issued at about 13° C. when the temperature of the room was between 23° and 31° C. The polariscope was maintained at 20° C. about an hour before polarizations were made, and the observation tube when inserted at other temperatures than 20° quickly came to this standard, a change of as much as two degrees taking place in four minutes.

Cane sugars polarized at temperatures higher than 20° C. are subject to a temperature correction which may be divided into two parts: (1) The temperature correction for pure sucrose, given by the formula $P. 20=P^t [1.+0.0003 (t-20)]$, and (2) the temperature correction as applied to pure levulose, the formula being $P. 20=P^t-0.00812 L (t-20)$. By properly applying the combination of these formulas to a raw-cane sugar polarized at temperatures other than 20°, it is possible to arrive at a result very close indeed to that which would be obtained when polarizing the sugars at 20°, as may be seen by the following set of observations on eight raw sugars, giving the polarizations actually obtained at 20° C. (the polariscope, the solution, and containers all being at this same degree), and polarizations of the same sugars at higher degrees (Table 1). These 95° sugars were assumed to contain 1.25 per cent invert sugar on the average, and having calculated the corrections for the sucrose and levulose separately and united all of these results, it was found that the corrected polarizations were only 0.0247 of a polariscopic degree lower than the polarizations actually made at 20° C.

Table 1.—*Comparison of calculated polarization of eight raw sugars with actual polarization at 20° C.*

Temperature (° C.) and polarization (° V.).		Difference in polarization.	Calculated correction—			Difference of calculated polarization from actual polarization at 20° C.
			For sucrose alone.	For levulose.	For both levulose and sucrose.	
At 20° C.:	At 26.5° C. ° V.:					
95.4	95.3	−0.10	+0.1868	−0.0330	+0.1538	+0.0538
At 21° C.:						
95.5	95.25	− .25	+ .1572	− .0279	+ .1293	− .1207
95.5	95.2	− .30	+ .1571	− .0279	+ .1292	− .1708
95.3	95.15	− .15	+ .1570	− .0279	+ .1291	− .0209
At 20° C.:						
95.3	95.15	− .15	+ .1855	− .0330	+ .1525	+ .0025
95.5	95.4	− .10	+ .1862	− .0330	+ .1532	+ .0532
95.3	95.2	− .10	+ .1855	− .0330	+ .1525	+ .0525
95.2	95.0	− .20	+ .1856	− .0330	+ .1526	− .0476
Average						− .0247

As the invert sugar content of these sugars was not definitely known, a further investigation of 12 samples of raw sugar was made in which the invert sugar content of each was accurately determined, giving the results shown in Table 2.

Table 2.—*Further comparison of calculated and observed polarizations on twelve samples of raw sugar whose invert sugar content was known.*

Temperature (° C.) and polarization (° V.).		Difference in polarization.	Calculated correction for sucrose alone.	Invert sugar.	Calculated correction for levulose alone.	Combined corrections.	Difference of calculated polarization from polarization at 20°.
At 20°:	At 28°:						
95.25	95.1	−0.15	+0.2282	1.33	−0.0432	+0.1850	+0.0350
85.80	85.7	− .10	+ .2057	2.50	− .0812	+ .1245	+ .0245
94.25	94.0	− .25	+ .2256	1.45	− .0471	+ .1785	− .0715
94.3	94.1	− .20	+ .2258	1.39	− .0451	+ .1807	− .0183
	At 25°:						
93.5	93.3	− .20	+ .1400	1.84	− .0374	+ .1026	− .0974
94.7	94.55	− .15	+ .1418	.81	− .0164	+ .1254	− .0246
85.8	85.7	− .10	+ .1286	1.77	− .0359	+ .0927	− .0073
94.7	94.6	− .10	+ .1419	1.07	− .0217	+ .1202	+ .0202
94.6	94.4	− .20	+ .1416	1.45	− .0294	+ .1122	− .0878
	At 27°:						
87.6	87.6	− .00	+ .1839	2.15	− .0595	+ .1244	[1]− .1244
95.3	95.15	− .15	+ .2001	.91	− .0260	+ .1741	+ .0241
94.6	94.4	− .20	+ .1998	1.48	− .0421	+ .1577	− .0423
First average							− .0101
Second average							− .0205

[1] Excluded from second average.

Having applied the formulas for correction of sucrose and levulose, the results obtained in the last column show the differences of the polarizations made at high temperatures and calculated back to 20° from the polarizations actually made at that temperature. The average of 12 such determinations gives a result for the sugars polarized above 20° of only 0.0101 polariscopic degrees lower than the polarizations actually made at 20° C. Omitting the tenth sample in this series, which seems to be irregular, we find that the average calculated polarization is only 0.0205 polariscopic degrees lower than the polarization actually made at 20°. These observations are quite in accord with those made by Browne, on mixtures containing known amounts of sucrose and levulose in raw sugars and molasses and reported by him in the article above cited.

In order to get a further comparison between polarizations made at 20° C. and those made at higher temperatures and calculated at 20° C., I have made monthly comparisons between the polarizations of many hundreds of samples tested both at the Sugar Trade Laboratory and at other laboratories.

By use of the formulas above given, the polarization of all the raw sugars coming to two technical laboratories have been arranged in groups covering monthly periods, corrected for temperature and compared with the corresponding average polarizations obtained in the Sugar Trade Laboratory when tested at 20° C.

The results found are expressed in the following table, which gives in decimal fractions of a single polariscopic degree the variations of my Laboratory "A" and Laboratory "B" results, after calculating to 20° C., from the results obtained in the Sugar Trade Laboratory when conducting the whole operation at 20° C.

Table 3.—*Variations in two laboratories where results were corrected for temperature from Trade Laboratory results obtained at 20° C.*

Date.	Corrections for Laboratory "A."			Difference from Trade Laboratory.	Corrections for Laboratory "B."			Difference from Trade Laboratory.
	Sucrose.	Levulose.	Total.		Sucrose.	Levulose.	Total.	
1911.								
March	+0.0800	−0.0140	+0.0660	−0.0070	+0.0424	−0.0059	+0.0365	−0.0096
April	+ .1290	− .0236	+ .1054	+ .0140	+ .0239	− .0036	+ .0203	+ .0200
May	+ .2002	− .0303	+ .1699	+ .1190	+ .0990	− .0180	+ .0810	+ .0710
June	+ .1977	− .0575	+ .1402	+ .0436	+ .1832	− .0355	+ .1477	+ .0117
July	+ .2235	− .0576	+ .1659	+ .0273	+ .2373	− .0580	+ .1793	− .0170
August	+ .2329	− .0634	+ .1695	− .0625	+ .1177	− .0344	+ .0833	+ .0193
September	+ .1379	− .0373	+ .1006	+ .0047	+ .1666	− .0620	+ .1146	− .0267
Average corrections for both laboratories					+ .1486	− .0351	+ .1135	+ .0148

Thus we see that the average discrepancy in this series of about 1,000 samples is only +0.0148 polariscopic degree, but if the levulose correction had not been applied this quantity would be increased to +0.0499.

The levulose correction is thus twice as great as the total experimental error and should by no means be ignored. The ideal method is, of course, to conduct the entire operation of polarizing sugars at 20° C., but as this requires an expensive plant or somewhat increased work and time by the method outlined, cases will arise where a temperature correction will be required. My contention is that if any such correction is to be applied it should be done in accord with a full appreciation of the significance of levulose as well as of sucrose and thus be made as correct as our present knowledge will allow.

REPORT OF COMMITTEE B ON RECOMMENDATIONS OF REFEREES.

By E. M. Chace, *Chairman.*

(Dairy products, foods and feeding stuffs, sugar, tannin, and medicinal plants and drugs.)

Foods and Feeding Stuffs.

It is recommended—

(1) That the method for acidity suggested by the referee for provisional adoption, together with the method of reporting results, be studied for another year. (See pp. 198, 201.)

Adopted.

(2) That the petroleum ether methods for fat, of the Cotton Seed Crushers Association, as given in the report of the referee, be printed and studied further. (See p. 198.)

Adopted.

(3) That the study of the modification of the official method for crude fiber be discontinued.

Adopted.

(4) That the study of the proper factor for converting nitrogen into protein be continued.

Adopted.

SUGAR.

It is recommended—

(1) That Herles's solution and neutral lead acetate be adopted provisionally as clarifying agents in polarizing cane products.

Finally adopted as provisional.

These methods have been formulated as follows:

Neutral lead acetate.—Prepare a saturated solution of normal lead acetate and, as in the case of the basic lead acetate solution, add it to the sugar solution before completing to volume. Its use is imperative when determining the reducing sugars in the solution used for polarization.

Basic lead nitrate (*Herles's solution*).—(1) Dissolve 250 grams of lead nitrate in water and make up to 500 cc. (2) Dissolve 25 grams of sodium hydroxid in water and make up to 500 cc.

Add equal amounts of the two solutions (1) and (2) to the sugar solution, shake, and add more if sufficient precipitation has not occurred, taking care not to add an excess. Then complete the volume with water. When this solution is used for clarification, the factor in the Clerget determination becomes 143.5 instead of 142.66.

(2) That the provisional method for the determination of moisture in molasses by the refractometer be amended so as to provide for the use of a pure sucrose solution instead of water for diluting samples too dark to read. (Bul. 132, p. 178.)

Adopted.

(3) That to the official aerometric method for the determination of total solids (Bul. 107, Rev., p. 65) a note be added to the effect that the results on molasses and other materials containing large amounts of invert sugar or nonsugar solids are only roughly approximate.

Approved and referred for final adoption in 1912.

(4) That it be noted that Horne's dry lead clarification method is not an optional method but is provisional and has the same standing as the wet clarification method. (Bul. 132, p. 189, and Bul. 107, Rev., p. 40.)

Adopted.

This method reads as follows:

Dry lead subacetate (*Horne's method*).—This clarifying agent is obtained as a dry powdered salt and should contain 72.8 per cent of lead, which corresponds to a composition of $3Pb(C_2H_3O_2)_2 2PbO$. Dissolve the normal or half-normal weight of the sugar solution in a flask with water and complete the volume. Add a small quantity of the dry salt and shake, then add more and shake again, repeating until a point of sufficient precipitation is reached to allow of an easy reading. An excess is to be avoided. Of this salt 0.1346 gram is equivalent to 1 cc of the solution of subacetate described under lead subacetate solution. When molasses or any other substance producing a heavy precipitate is being clarified, some dry, coarse sand should be added to break up the balls of lead subacetate and precipitate. (This method is to have equal weight with the use of a solution of lead subacetate in clarifying cane, sorghum, and beet products.)

(5) That the referee's recommendation as to future study be adopted, i. e., that further work should be done on the determination of moisture in sugars by means of the refractometer, on the influence of the composition of the basic lead acetate (as clarifying agent) on the polarization of sugar solutions, and on the factors used in the formula for determining commercial glucose.

Adopted.

TANNIN.

It is recommended—

(1) That the methods for the analysis of leather proposed for provisional adoption by the referee be printed with the view to subsequent adoption (see page 231).

Adopted.

MEDICINAL PLANTS AND DRUGS.

It is recommended—

(1) That the recommendation of the associate referee as to further study of methods for mixtures of acetphenetidin and salicylates be adopted.

Adopted.

(2) That the following minor changes be made in the provisional method for caffein (Bul. 132, p. 197): That the size of the flasks in lines 3, 13, and 23 be changed from 100 cc to 200 cc; that in line 11 "Add 4 times the volume, in this case 80 cc" be changed to "Add 3 times the volume, in this case 60 cc"; that in line 15 "for a second extraction" be changed to "for a second and third extraction."

Approved and referred to association for final adoption in 1912.

(3) That the work on medicated soft drinks be continued.

Adopted.

(4) That the work on medicinal plants and drugs be continued.

Adopted.

REPORT OF THE AUDITING COMMITTEE.

The auditing committee has examined the accounts of the treasurer and find them to be correct.

[Signed] J. M. BARTLETT, *Chairman.*
BURT L. HARTWELL.[1]

The report of the treasurer for 1911 was, in brief, as follows, detailed statement and vouchers being filed with the records of the secretary:

RECEIPTS.

Balance from 1910	$43.47
Dues from 40 stations, colleges, State chemists, etc., of $2 each	80.00
Total	123.47

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[1] O. M. Shedd, the third member of the committee, was not present when the report was presented

EXPENDITURES.

December 20, 1910, to the Nittany Printing and Publishing Co. (for printing of food standards committee, as ordered by the association)	$43.47
January 30, 1911, 400 circulars and 250 envelopes (dues and collaboration)	7.25
Postage on same	3.00
August 30, 1911, 550 circulars and 200 envelopes (call for meeting and program)	14.25
Postage on same	2.18
Miscellaneous postage	1.14
Total	71.29
Balance	52.18

NOVEMBER 13, 1911.

REPORT OF THE COMMITTEE ON RESOLUTIONS.

The following report was submitted by William Frear, as chairman of the committee on resolutions:

Resolved, That this association hereby expresses its appreciation to President Woll for the able, impartial, and courteous manner in which he has presided over its deliberations.

Resolved, That the association hereby thanks the secretary of the association, Dr. H. W. Wiley, for his continuance of the service to which the association so largely owes its vigor and efficiency.

Resolved, That this association is grateful to the Secretary of Agriculture for his renewed expression of encouragement to, and assurance of cooperation with, the association in its work.

Resolved, That this association hereby expresses its high appreciation of the courtesies extended to its members by the Cosmos Club and the Washington Section of the American Chemical Society.

Resolved, That the secretary of the association be instructed to communicate to the parties and organizations concerned copies of the foregoing resolutions.

Adopted.

Mr. Haskins called attention to the fact that no report had as yet been made by the special committee appointed in 1909 to study the availability of phosphoric acid in basic slag phosphate by means of vegetation experiments; and inasmuch as the work was of great importance it was moved that the committee be discharged and a new committee appointed. The motion was carried. (See p. 252 for personnel of committee.)

REPORT ON MEAT AND FISH.

By RALPH HOAGLAND, *Associate Referee.*[1]

INTRODUCTION.

In order to get the opinion of various food chemists as to what problems in connection with the adulteration of meat and fish seemed worthy of investigation, letters were sent out early in the summer to a number of chemists asking for suggestions. The following problems were proposed:

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[1] Presented by A. S. Mitchell.

(1) Determination of starch in sausage.

(2) Detection of incipient putrefaction in meats.

(3) Determination of tin in canned goods.

All three of these problems need attention, but the third was selected for cooperative work.

Mayerhofer's method for the determination of starch in sausage, as given in Bulletin 107, Revised, Bureau of Chemistry, is tedious, inaccurate, and generally unsatisfactory, as the writer has learned from several months' experience with the method. A more rapid and accurate method for the determination of starch in meat products is much needed, owing to the widespread use of starch in sausages.

The second problem, that of methods for the detection of incipient putrefaction in meat products, is of great importance, but demands long-continued investigation by those particularly fitted for that kind of work, rather than cooperative work, at this time.

The Federal Board of Food and Drug Inspection has placed a limit of 300 mg of tin per kilo in canned goods; hence the importance of a rapid and accurate method for determination of tin in such products.

R. E. Doolittle, then in charge of the Food and Drug Inspection Laboratory, New York City, suggested the problem and also the method which has been used in the cooperative work. He stated that he had found the method for determination of heavy metals in canned goods, Bulletin 107, Revised, tedious and unreliable, as have also Schreiber and Taber. (See Circular 67, Bureau of Chemistry.) Doolittle also states that he has found Schreiber and Taber's sulphuric acid digestion method, as described in the circular mentioned, rather too time consuming, and needing too much personal attention for inspection work.

The method proposed by Doolittle and Lourie, and which they state has given excellent satisfaction in routine work, was incorporated in the following letter sent out to cooperators:

INSTRUCTIONS FOR COLLABORATIVE WORK.

The determination of tin in canned food products (Doolittle and Lourie).— Place 25 to 50 grams of the well-mixed and finely ground sample in a Kjeldahl flask (800–1,000 cc) and add 25 to 50 cc of concentrated sulphuric acid, the amount depending upon the weight of the charge. Place the flask on a hot plate or on wire gauze over free flame; add about 30 cc of concentrated nitric acid, raise temperature to boil, and heat till white fumes are generated, then without cooling add 10 cc of nitric acid and continue heating as before. Repeat the nitric acid addition until the solution remains clear (usually straw color) after boiling off the nitric acid fumes. The digestion can easily be accomplished in three hours with from three to four additions of the acid. Let the solution cool and dilute to about 400 cc with water. Nearly neutralize with concentrated ammonium hydroxid, transfer the solution to a beaker, saturate with hydrogen sulphid, and let the precipitate settle on a steam bath. Filter, wash the precipitate with a little hot water saturated with hydrogen sulphid, and then dissolve it in hot yellow ammonium sulphid; reprecipitate with acetic acid or hydrochloric acid, filter on ashless paper, ignite and weigh as stannic oxid (SnO_2). Finally moisten with nitric acid. (NOTE.—50 cc of concentrated ammonium hydroxid will nearly neutralize 25 of concentrated sulphuric acid. Make the usual tests for the complete precipitation in the filtrate from the first tin sulphid precipitate. In the case of canned vegetables as high as 100 grams may be taken without using more than 50 cc of sulphuric acid. With fish it is best to use as many cubic centimeters of sulphuric acid as grams of fish. The rapidity of the digestion depends on the temperature maintained—the higher the temperature the faster the material is oxidized.)

Samples to be analyzed.—A sample of stannous chlorid is being mailed you under separate cover for use in this work.

All determinations are to be made in *duplicate*, and individual as well as average results should be reported.

1. Weigh into three Kjeldahl flasks, respectively, 20, 30, and 50 mg of the stannous chlorid, and then to each flask add 50 grams of finely ground fresh meat; proceed with the determination as directed.
2. Procure approximately 1-pound cans of the following products: roast beef, rhubarb, pumpkin.

Preparation of sample.—In the case of meat run the product twice through a meat grinder and then mix thoroughly with the liquid portion. In the case of rhubarb macerate the solid portion and make a uniform mixture with the juice.

Use 50 grams of each product for the determination. In case a very small amount of tin is obtained repeat the operation, using a larger quantity of material.

If the products mentioned are not obtainable, get similar products which will include a meat, fruit, and vegetable. * * *

ANALYTICAL RESULTS.

Results obtained by the nitric-acid method when known amounts of tin were added to meat.

Analyst.	Stannous chlorid added.	Stannic oxid added.	Stannic oxid recovered.	Average.
	Mg.	*Mg.*	*Mg.*	*Mg.*
H. Schreiber and W. C. Taber, Washington, D. C.........	20	10.6 10.6	10.6 10.5	10.55
	30	15.9 15.9	14.8 15.8	15.3
	50	26.5 26.5	25.4 25.3	25.35
H. L. Lourie,[1] New York City..........................	20	9.9 9.9	9.6 9.6	9.6
	30	14.8 14.8	15.3 14.4	14.85
	50	24.6 24.6	25.0 24.3	24.65

[1] Did not add exactly 20, 30, and 50 mg of tin salt; calculated to comparable basis.

Mr. Lourie's analysis of the tin salt showed 49.3 per cent of stannic oxid and 38.85 per cent of tin. Schreiber and Taber's analysis gave 53 per cent of the oxid.

Schreiber and Taber made an interesting comparative study of the nitric-acid method (Doolittle and Lourie) and the sulphuric-acid method (Schreiber and Taber) for the determination of tin in various products, with the following results:

Results by both methods when known amounts of tin salt were added to 50 grams of corned beef (Schreiber and Taber).

Stannic oxid added.	Stannic oxid already in beef.[1]	Total stannic oxid calculated as present.	Total stannic oxid found.
Gram.	*Gram.*	*Gram.*	*Gram.*
0.0132	0.0048	0.0180	[1]0.0189
.0134	.0048	.0182	[1].0198
.0124	.0048	.0172	[2].0179
.0117	.0048	.0165	[2].0161

[1] Sulphuric acid method.

[2] Nitric acid method.

Results by Schreiber and Taber on tin in various commercial canned products, by both methods.

[Expressed in grams of tin per 100 grams of sample.]

Sample.	Nitric acid method.		Sulphuric acid method.	
Rhubarb	0.0469 .0488	0.0479	0.0492 .0494	0.0493
Gooseberries	.0223 .0220	.0222	.0254 .0281	.0267
Strawberries	.0217 .0225	.0221	.0198 .0243	.0221
Pumpkin	[1].0569 [1].0570	.0570	.0560 .0500	.0530
Mustard sardines	[1].1544 [1].1723	.1634	[1].1571 [1].1563	.1567
Mustard sardines plus sodium chlorid	[1].1620 [1].1481	.1550	[1].1505 [1].1652	.1578

[1] 50-gram sample used in these determinations; all others were 100-gram samples.

Lourie reports the following results of analyses of canned goods for tin at the New York Food and Drug Inspection Laboratory by the nitric-acid method, unless otherwise noted:

Tin found in various types of canned goods in routine work in New York Laboratory.

Sample No.	Substance.	Amount of tin found.
		Mg per kilo.
N. Y. 21386	Tomato herring	183.0
N. Y. 21387	....do	105.0
N. Y. 21389	....do	446.0
N. Y. 21410	....do	189.9
N. Y. 21411	....do	551.0
N. Y. 21427	....do	217.0
N. Y. 21428	....do	169.0
N. Y. 21264	....do	407.4
N. Y. 21266	....do	134.7
N. Y. 21267	....do	494.9
N. Y. 21268	....do	116.0
N. Y. 21269	....do	116.0
N. Y. 21280	....do	472.0
Check [1]	....do	340.4
Check [1]	....do	327.8
Check [2]	....do	275.1
N. Y. 24783	....do	539.4
N. Y. 24783 [1]	....do	554.8
N. Y. 21855	....do	255.0
N. Y. 21836	....do	58.3
N. Y. 21289	Herring	235.0
N. Y. 21290	....do	96.0
N. Y. 21291	....do	78.8
N. Y. 21244	....do	285.0
N. Y. 21245	....do	278.0
N. Y. 21246	....do	539.0
N. Y. 21247	....do	523.0
N. Y. 21592	....do	312.0
N. Y. 21580	....do	60.0
N. Y. 21579	....do	121.0
N. Y. 21876	....do	61.0
N. Y. 21783	Kippered herring	504.0
N. Y. 21782	Soused mackerel	66.2
N. Y. 21861	W. I. spiny lobster	51.2
N. Y. 21443	Apricot pulp	157.0
N. Y	Sardines	530.0
N. Y. 27384	Mixed vegetables	230.0
I. S. 3446	Apple butter	677.8
N. Y. 21390	Cockles	5.0
N. Y. 21443	Sardines in tomato sauce	443.0
N. Y. 21487	....do	654.0
N. Y. 21243	Sorrel	70.0
N. Y. 24429	Herring in bouillon, can 1	528.0
	Herring in bouillon, can 2	506.0
N. Y. 24582	Vegetables in vinegar	104.8
N. Y. 24738:		
No. 1 [2]	Herrings in bouillon	219.1
No. 1 [1]	....do	409.8
No. 2 [1]	....do	892.8
No. 2 [1]	....do	819.6
No. 2 [2]	....do	434.2

[1] Oxidation method.

[2] Munson method.

COMMENTS ON RESULTS.

The results of the cooperative work on Lourie's method for the determination of tin in canned goods are quite satisfactory, duplicate determinations checking well, while the results of the two cooperators check closely, considering the comparatively small quantities of tin which are recovered.

Schreiber and Taber find that results by the sulphuric-acid and the nitric-acid (Lourie) methods agree very closely.

I have tried out Lourie's method in a preliminary way and find that with constant attention complete digestion of 50 grams of meat can be accomplished in a little over two hours. Doubtless the time for digestion of other materials will vary one way or the other. After the complete digestion of the material one has to observe only the precaution necessary for the determination of tin.

Schreiber and Taber have the following additional comments to make regarding Doolittle and Lourie's method for the determination of tin in canned goods:

Schreiber and Taber: From results of our work it would seem that the nitric acid method gives as good results as the sulphuric acid digestion method, and that it recovers all the tin in the sample.

Some of the disadvantages of the proposed nitric acid method might be mentioned. The noxious oxids of sulphuric acid and organic matter form a very disagreeable feature. Then it was found that meats or fish require for their complete digestion, instead of the three or four additions of nitric acid as mentioned in the method, at least twice that number. It was also found in our work that it is not safe to add the nitric acid to the hot mass in the flask, as stated in the method, as there is great danger of frothing, and the sample running out of the flask, especially in the first stages of the digestion.

We believe that ammonium sulphid is not the best solvent to use in dissolving the tin sulphid. When the solvent is used, the subsequent neutralization of the solution is slow and tedious, as the exact strength of the sulphid employed is unknown, and the solution must not be made too acid in precipitating the tin or some will be lost. Again large quantities of sulphur are thrown down which become troublesome in the subsequent filtration, running through the filter, so that one can not tell whether or not a loss of the precipitate is taking place.

In our method for tin determination given in Circular 67, Bureau of Chemistry, potassium hydroxid (20 grams in 100 cc of water), was used as a solvent for a tin sulphid. This we have found to give as accurate results besides permitting the accurate and quick neutralization of a solution for the precipitation of the tin; it also does away with the large sulphur precipitate.

In the determinations given, potassium hydroxid was used to dissolve the tin sulphids in the sulphuric acid method and ammonium sulphid in the nitric acid method.

Concerning the time of digestion, an accurate account was kept for the two methods. In twelve determinations given in Table 3, those run by the nitric acid method were all finished in two hours; those by the sulphuric acid method, in four and one-half hours. In the second set of 12 digestions, those by the nitric acid method were finished in four hours, while those by the sulphuric acid method required four and one-half hours. On many materials it seems that the nitric acid method is shorter, but one must take into consideration that the nitric acid method requires almost continuous attention in adding more acid until the digestion is complete, while with the sulphuric acid method, after the frothing stage has passed, the sample needs no more attention.

It would seem that no one method is best for all materials and under all circumstances. The nitric acid digestion method has been used with apparent success in the laboratory.

RECOMMENDATION.

I would respectfully recommend that the associate referee on meats and fish for the next year conduct a study of the nitric and sulphuric acid methods for determination of tin in canned goods, and if results warrant, recommend one of these methods for adoption by the association as a provisional method.

REPORT ON FRUIT AND FRUIT PRODUCTS.

By A. W. Blair, *Associate Referee.*

INTRODUCTION.

The work on fruit and fruit products for this year was again confined to moisture determinations. Samples were prepared from green oranges (varying in diameter from 1½ inches to 2½ inches) as follows: Whole oranges were sliced, run through a meat chopper, and thoroughly mixed; portions were then weighed into tin-lead dishes, and dried in the air oven at 50° to 60° C. for about fifteen hours to preserve for shipping.

Chemists representing seven laboratories offered to cooperate. Five portions of the partially dried oranges, with weight of container and sample, and net weight of sample (both weights refer to the fresh sample immediately after it was prepared) together with instructions, were sent to each laboratory. Three methods were proposed as follows:

I. Water-jacketed oven: Dry in water-jacketed oven for 30 hours, or longer if necessary, weighing at the end of each six hours; report weights for each drying, and percentage for final drying. (Bul. 122, pp. 219–220).

II. Vacuum oven at 70° C.: Dry to constant weight in vacuum oven at 70° C. State length of time required. (Bul. 132, p. 150.)

III. Dry to constant weight in vacuum over c. p. sulphuric acid, without the aid of heat; state time required. (Bul. 137, pp. 138–140.)

ANALYTICAL RESULTS.

Five laboratories submitted results which are reported in the following table:

Comparison of three methods for determining moisture in fruit.

Method and analyst.	Net weight fresh sample.	Weight of container and fresh sample.	Weights on consecutive dryings.												Total loss in drying.	Moisture.	Average.
			1	2	3	4	5	6	7	8	9	10	11	18			
(A) Water jacketed oven:	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*P. ct.*	
A. W. Blair	(1) 25.8911	39.6598	18.3725	18.2540	18.2010	18.1712	18.1523	......	......	......	......	......	......	......	21.5075	83.07	
	(2) 25.7787	38.9569	17.7488	17.6265	17.5703	17.5390	17.5205	......	......	......	......	......	......	......	21.4364	83.16	83.14
	(3) 23.8697	34.9125	15.2515	15.1445	15.0956	15.0699	15.0523	......	......	......	......	......	......	......	19.8602	83.20	
S. E. Collison	22.7803	34.2008	15.3673	15.3289	15.3005	15.2804	15.2625	15.2509	......	......	......	......	......	......	18.9499	83.18	83.18
									18 hrs.	18 hrs.							
E. O. Eaton	(1) 25.3069	39.3798	18.5230	18.4190	18.3750	18.3510	18.3340	18.3160	18.2850	18.2770	18.2740	......	......	......	21.1058	83.40	
	(2) 24.8779	37.9969	17.5225	17.4200	17.3775	17.3500	17.3330	17.3160	17.2900	17.2800	17.2785	......	......	......	20.7184	83.27	
	(3) 26.4443	39.7402	17.8625	17.8510	17.8010	17.7745	17.7500	17.7320	17.7000	17.6920	17.6900	......	......	......	22.0502	83.38	83.33
	(4) 24.4899	36.8555	16.6600	16.5615	16.5250	16.5005	16.4830	16.4660	16.4400	16.4300	16.4275	......	......	......	20.4280	83.42	
	(5) 25.2106	38.4854	17.7980	17.6740	17.6260	17.6000	17.5800	17.5630	17.5300	17.5230	17.5205	......	......	......	20.9649	83.16	
H. C. Gore	(1) 23.8808	35.3370	15.6800	15.6046	15.5563	15.5285	......	......	......	......	......	......	......	......	19.8085	82.95	82.93
	(2) 22.2843	33.8475	15.5185	15.4468	15.4022	15.3720	......	......	......	......	......	......	......	......	18.4755	82.91	
A. M. Henry	(1) 24.3231	36.4145	16.3980	16.2770	16.2390	16.2100	16.1835	16.1600	16.1400	16.1322	16.1297	......	......	......	20.2848	83.40	83.22
	(2) 20.3369	32.5680	15.8825	15.8110	15.7735	15.7475	15.7275	15.7095	15.6915	15.6830	15.6820	......	......	......	16.8860	83.03	
H. D. Poore	24.3452	36.0292	15.9446	15.8494	15.8193	15.7960	15.7679	15.7517	15.7457	15.7319	15.7258	15.7173	15.7088	15.6695	20.3597	83.62	83.62
(B) Vacuum at 70° C.:																	
H. D. Poore	23.8874	35.8300	16.3598	16.3222	16.2868	16.2671	16.2615	16.2397	16.2190	16.2091	16.2008	16.1972	[1] 16.1916	......	19.6384	82.21	82.21
H. C. Gore	(1) 21.1118	32.0440	14.8474	14.8022	14.7793	14.7620	......	......	......	......	......	......	......	......	......	81.86	81.79
	(2) 22.3681	37.7305	19.5380	19.4894	19.4653	19.4485	(Intervals about 6 hours.)				......	......	......	......	......	81.73	
(C) Vacuum over sulphuric acid without heat:																	
H. D. Poore	23.6912	35.2580	15.9709	15.9502	15.9263	15.9148	15.9104	15.9034	15.8988	15.8786	15.8745	[2] 15.8623	......	......	19.3957	81.86	81.86
A. M. Henry	(1) 24.5273	36.4550	16.6490	16.6205	[3] 16.5730	......	......	......	......	......	......	......	......	......	19.8820	81.06	80.98
	(2) 21.7885	37.0655	19.4995	19.4795	[3] 19.4385	......	......	......	......	......	......	......	......	......	17.6270	80.91	
H. C. Gore	20.2028	32.3085	15.8337	(20 days.)		......	......	......	......	......	......	......	......	......	......	81.55	81.55
(D) Atmospheric pressure; over calcium chlorid:																	
A. M. Henry	29.3263	40.4270	16.5480	(6 days.)		......	......	......	......	......	......	......	......	......	23.8790	81.42	81.42
(E) Electric oven at 100° C.:																	
A. W. Blair	(1) 24.0525	34.6445	14.8485	14.7683	14.7208	14.6870	14.6687								19.9758	83.05	82.98
	(2) 21.9358	32.6350	14.6104	14.5415	14.4960	14.4647	14.4470	(5 six-hour intervals.)			......	......	......	......	18.1880	82.92	
S. E. Collison	23.3652	36.9700	17.6994	17.6288	17.5795	17.5519	17.6255								19.4445	83.18	83.18

[1] Average intervals about 5¾ hours.

[2] Average intervals about 37 hours.

[3] Average intervals about 24 hours.

The results with the water-jacketed oven do not agree as they should. This is partly due to the fact that no two analysts dried for the same length of time, and partly, no doubt, to slight decomposition of organic materials caused by long-continued heating. It will be observed that Mr. Eaton secured fairly concordant results on four out of five samples when all were dried for the same length of time, his greatest difference on the four being 0.13 per cent when about 25 grams of fresh material were used.

The results by the vacuum methods are decidedly lower than with the water oven and are even less concordant. It will be noted that Mr. Gore's results with vacuum at 70° C. and vacuum over sulphuric acid, without the aid of heat, agree within reasonable limits, and are also in fair agreement with Mr. Poore's work by the last-named method. The fact that in most cases duplicate determinations agree within the limit of error would indicate that lack of agreement with a given method is due to differences in the time of drying.

Tests made with the electric oven at 100° C. show results in fair agreement with those obtained with the water oven. It is quite evident that the water oven, and also the electric oven, give results that are too high; that is, there is undoubtedly some loss through decomposition. This probably holds true for all fruits and fruit products containing much sugar, and points to the necessity of adopting some other method, when accurate results are desired.

Mr. Gore suggests the use of lime or barium oxid as a desiccating agent instead of sulphuric acid, to avoid the presence of the vapors of the latter in the desiccator.

It is suggested that the vacuum methods be further tested and that an effort be made to have all the work done under as nearly the same conditions as possible.

REPORT OF COMMITTEE ON NOMINATIONS.

In the absence of Mr. L. L. Van Slyke, chairman of the nominating committee, Mr. W. A. Withers presented the following report:

The following nominations are made for officers for the coming year:

President, H. J. Patterson, College Park, Md.; vice president, G. S. Fraps, College Station, Tex.; secretary, H. W. Wiley, Washington, D. C.; additional members of the executive committee: R. E. Doolittle, Washington, D. C.; A. J. Patten, East Lansing, Mich.

The secretary was instructed to cast the unanimous vote of the association for the officers as nominated.

In accordance with the resolution ordering that the referees and associates should be appointed by the outgoing executive committee rather than by the incoming committee, the president here announced the appointments made. These are given on page 251, with such subsequent changes as were necessitated by declinations, etc.

REPORT ON TANNIN.

By J. S. ROGERS, *Referee.*

PLAN OF WORK AND DESCRIPTION OF SAMPLES.

The work for this year has been designed to study certain conditions of three phases of leather analysis: (1) Shortening the time required for the extraction of fats; (2) a comparison of three methods of extraction of the soluble materials (other than fats) in leather; (3) experiments to show that basic lead acetate, when used as a clearing agent for sugar solutions in leather analysis, removes large amounts of sugar actually present.

The two samples of leather sent out were prepared as follows:

No. 1, an oak-tanned sole leather, was finely ground, allowed to come to air-dry condition, and thoroughly mixed. Moisture determinations were made and sugar was determined on the extract. A known amount of grape sugar in solution was then added to and mixed with the weighed ground leather. The mixing was continued under the blast of an electric fan until the leather was nearly dry, after which it was spread out in a thin layer to come to air-dry condition. After several days' drying in the air, the sample was reground, again allowed to come to air-dry condition, thoroughly mixed, and a moisture determination was made. Knowing the per cent of moisture and the per cent of sugar originally present, the per cent of moisture after the sugar was added, the original weight of the leather, and the weight of the sugar added, it was possible to calculate the per cent of sugar present in the prepared sample. The sugar originally present was 0.60 per cent. The calculated amount added was 7.73 per cent, giving 8.33 per cent total sugar in the prepared sample.

No. 2, an oak-tanned, heavily greased harness leather, was finely ground, spread out in a thin layer, and allowed to come to an air-dry condition.

Samples No. 1 and No. 2, as soon as prepared, were put up in quart Mason jars and tightly stoppered.

DIRECTIONS FOR LEATHER WORK.

FAT EXTRACTION.

Place 15 grams of the sample as received in an S & S thimble and extract in a Soxhlet extractor, using petroleum ether, distilling between 50° and 80° C., as directed under A, B, C, and D. Heat at such a rate that the liquid siphons approximately four times per hour.

(A) Extract two portions, 15 grams each, for 24 hours; save the leather residue; evaporate the ether from the extract on a steam bath, and dry to constant weight in a water oven; weigh and calculate the per cent of fat present. (A should be completed and the amount of fat determined before B, C, and D are started.)

(B) Extract two portions of 15 grams each for 6 hours.

(C) Extract two portions of 15 grams each for 8 hours.

(D) Extract two portions of 15 grams each for 10 hours.

Save the leather residues from B, C, and D, determine the fats in each extract as in A, and if the fat has not all been extracted in the stated time continue until the extraction is complete, noting in each case the time required and the color, consistency, and odor, and general appearance of the fat residue.

Reserve the leather residues from the fat extraction for the extraction of the uncombined tannins and nontannins, bringing them to an air-dry condition before this extraction is begun.

1. Place the two residues of A together and the two residues of B together. Use these as duplicates for the extraction by the A. L. C. A. method (E).
2. Take the two residues from C and use these as duplicates for the extraction by the method of the Bureau of Chemistry (F).
3. Take the two residues from D and use as duplicates for the extraction by the alcohol method (G).

EXTRACTION OF UNCOMBINED TANNINS AND NONTANNINS.

E. *Method of A. L. C. A.*—Digest the leather residues A and B as duplicates in percolators overnight, transfer the extract to a 2-liter flask, then extract with water at 50° C. for three hours, removing the extract from a side tube of the extractor, thus avoiding boiling the extract. The total volume of solution to be 2 liters. Determine the glucose, soluble solids, and nontannins as directed below:

F. *Method of the Bureau of Chemistry.*—Take the leather residues from C and moisten thoroughly with distilled water. Transfer leather and water to a Soxhlet extractor and place cotton above and below the leather in the extractor, to prevent it from being carried over at the time of siphoning. The cylinder of the extractor shall be surrounded by water bath kept at 50° C. At the beginning of the extraction pour 250 cc of distilled water (including that used in moistening the leather) into the Soxhlet and allow it to siphon into the flask below, then begin the boiling. At the end of the first hour remove the flame and transfer the extract to a 1-liter graduated flask, add 200 cc of distilled water to the Soxhlet and continue the extraction for two hours. Remove the extract, add 200 cc of water and continue the extraction for five hours and remove the extract. Make one more extraction, adding 200 cc as before, and continuing the extraction for six hours. This gives 14 hours' extraction and an extract that does not exceed 1 liter in volume. Make the extract up to 1 liter at room temperature, and use as directed below for the determination of glucose, soluble solids, and nontannins.

G. *Alcohol method.*—Take the two leather residues from D (they may be extracted in the same thimble in which the ether extraction was made after they come to an air-dry condition) and extract with 95 per cent alcohol for eight hours in a Soxhlet extractor. Add 250 cc of water to the extract and evaporate the alcohol on the steam bath, make the solution to 1 liter, at room temperature, and determine glucose, soluble solids, and nontannins as directed below.

DETERMINATION OF GLUCOSE.

(*a*) To 200 cc of the extract add 25 cc of a saturated solution of normal lead acetate, mix thoroughly, allow to stand about 15 minutes and filter. The funnels and beakers must be kept covered to prevent evaporation. Remove the lead from the filtrate by adding a slight excess of solid potassium oxalate and filtering. Pipette 150 cc of the last filtrate into a 600 cc Erlenmeyer flask and add 5 cc of concentrated hydrochloric acid and boil under a reflux condenser for two hours, cool, neutralize with anhydrous sodium carbonate, transfer to a 200-cc graduated flask, and make to volume. Filter through a double filter. (This filtrate must be clear.) Use 50 cc of this filtrate for the determination of sugar.

Copper-sulphate solution.—Dissolve 34.639 grams of anhydrous copper sulphate in water and dilute to 500 cc.

Alkaline tartrate solution.—Dissolve 173 grams of Rochelle salts and 125 grams of potassium hydroxid in water and dilute to 500 cc.

Place 30 cc of the copper solution, 30 cc of the alkaline tartrate solution, and 35 cc of water in a beaker and heat to boiling. Add 50 cc of the solution of the material containing the sugar, which must be so prepared as not to contain more than 0.25 grams of dextrose, and boil for two minutes. Filter immediately through asbestos without diluting, wash with hot water, alcohol, and finally with ether, dry for half an hour in a water oven, and weigh as cuprous oxid; determine the amount of dextrose by the use of Allihn's table (Bul. 107, Rev., p. 50).

(*b*) Determine sugar in the same manner as in (*a*), except that instead of using 25 cc of saturated neutral lead acetate, use 25 cc of saturated basic lead acetate.

SOLUBLE SOLIDS.

To 2 grams of kaolin in a beaker add 75 cc of extract, stir, let stand for 15 minutes, decant and discard as much as possible of the supernatant liquid, and again add 75 cc of extract to the kaolin, stir and pour at once on a No. 590 S & S folded filter, keep the filter full and the funnel and receiving vessel covered, reject the first 150 cc of the filtrate, evaporate, and dry the next 100 cc (which must be brilliantly clear). Conduct the evaporation and drying in flat-bottomed glass dishes from 2^a to 3 inches in diameter, evaporate, and dry for 16 hours in a combined evaporator and drier from 98° to 100° C., or after evaporating dry for 12 hours on the bottom shelf of a water oven at from 98° to 100° C.

NONTANNINS.

A quantity of hide powder sufficient for the number of analyses to be made *during a day* shall be prepared in the following manner: Digest with 10 times its weight of water until thoroughly soaked. Add 3 per cent of chrome alum in solution, agitate by either shaking or stirring occasionally for several hours, and let stand overnight. Wash by squeezing through linen, continuing the washing until the wash water gives no precipitate with barium chlorid, squeeze the hide, using a press if necessary, so that the wet hide will contain between 70 and 75 per cent of water. Use approximately 20 grams of wet hide for moisture determination. Add to 200 cc of the original solution such quantity of wet hide as represents from 12 to 13 grams of dry hide, shake for 10 minutes in some form of mechanical shaker, and squeeze immediately through linen. Add 2 grams of the kaolin to the filtrate, stir and filter through folded filter S & S No. 590 of sufficient size to hold the entire filtrate, returning until clear, evaporating 100 cc of the filtrate. The weight of the residue must be corrected for the dilution caused by the water contained in the wet hide.

NOTE.—In order to limit the amount of dried hide powder used, determine the moisture in the air-dried hide powder and calculate the quantity equal to at least 15 grams of actual dry hide powder, take any multiple of this quantity, according to the number of analyses to be made, and after chroming and washing as directed, squeeze to a weight representing 70 to 75 per cent water. Weigh the whole amount and divide by the multiple of the grams of actual dry hide powder taken to obtain the weight of the wet hide powder for 200 cc of solution. *The hide powder should always be used the same day that it is washed.*

The nontannin filtrate must not give a precipitate with a 1 per cent gelatin, 10 per cent salt solution.

TANNIN.

The tannin content is shown by the difference between the soluble solids and the corrected nontannins.

ANALYTICAL RESULTS.

The results received from those cooperating are given in the following tables:

TABLE 1.—*The extraction of fat by petroleum ether.*

Analyst.	Address.	Sample No. 1.				Sample No. 2.			
		6 hours.	8 hours.	10 hours.	24 hours.	6 hours.	8 hours.	10 hours.	24 hours.
J. R. Blockey [1]	Leathersellers Co.'s Technical College, London.	2.07	2.10	2.05	2.16	28.80	29.00	29.30	29.40
		2.00	2.10	2.10	2.07	29.00	29.20	29.40	29.60
M. F. Nichols	Nichols's Laboratory, Grand Rapids, Mich.	2.04	2.09	2.08	2.09	29.33	29.10	29.72	29.74
		2.06	2.05	2.04	2.08	*30.00	29.30	*30.00	29.47
J. M. Seltzer	Kistler Lesh & Co., Lock Haven, Pa.	1.97	2.02	2.00	2.09	29.17	29.11	29.47	29.54
		1.95	2.00	1.98	2.07	29.13	29.13	29.35	29.40
J. S. Rogers	Bureau of Chemistry, Washington, D. C.	1.98	2.02	2.02	2.05	29.27	29.47	29.63	29.64
		1.97	1.98	2.04	2.06	29.39	......	29.63	29.67
B. J. Ray [2]	Agricultural Experiment Station, Raleigh, N. C.	2.03	1.99	2.15	2.04	29.25	*25.19	29.41	29.47
		1.97	......	2.10	2.06	......	......	......	29.30
P. F. Trowbridge	Agricultural Experiment Station, Columbia, Mo.	1.99	1.99	1.97	1.92	29.06	28.92	29.29	29.23
		2.17	1.90	1.99	1.99	28.78	*28.39	29.30	*28.92
T. A. Faust	Yocum-Eachus Laboratory, Newark, N. J.	2.01	2.12	2.02	*2.27	28.97	28.95	29.33	29.68
		2.07	2.17	2.19	*2.31	29.01	29.30	29.14	29.38
Averages		2.02	2.04	2.04	2.05	29.09	29.14	29.41	29.49

[1] The siphonings occurred from 6 to 8 times per hour.

[2] The result *25.19 is thought to be too low on account of the leather being too tightly packed in the extraction thimble.

NOTE.—Results marked with an asterisk are not included in the averages.

TABLE 2.—*Leather extraction and analysis of the extract.*

Analyst.	A. L. C. A. method.					Bureau of Chemistry method.					Alcohol method.				
	Soluble solids.	Non-tannins.	Tannins.	Glucose A[1].	Glucose B[2].	Soluble solids.	Non-tannins.	Tannins.	Glucose A[1].	Glucose B[2].	Soluble solids.	Non-tannins.	Tannins.	Glucose A[1].	Glucose B[2].
Sample No. 1.	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*
J. M. Seltzer	23.4	12.7	10.7	8.28	6.60	24.7	12.4	12.3	8.30	6.60	25.1	12.0	13.1	8.13	6.48
	23.0	12.7	10.3	8.42	6.72	24.0	12.2	11.8	8.46	6.68	25.6	12.6	13.0	8.20	6.52
J. S. Rogers	22.7	11.3	11.4	8.07	6.50	25.2	11.0	14.2	7.92	5.09	25.0	11.4	13.6	8.02	
	23.0	11.2	11.8	8.41	6.38	24.6	10.6	14.0	8.20	6.22	26.1	11.4	14.7	8.02	
B. J. Ray [3]	20.8			*.50		22.0			*6.13		23.9			*7.59	
	27.4			*.42		23.6			*3.96		24.6			*7.63	
J. R. Blockey [4]	24.5	12.2	12.3	8.60	7.40						*20.3	10.6	*9.7	6.05	4.35
	24.1	12.6	11.5	8.70	7.60						*20.4	10.6	*9.7	6.00	4.20
	24.4	12.8	11.6	8.70	7.58										
M. F. Nichols	19.2	11.6	*7.6			*17.3	10.6	*6.7			22.5	12.1	10.4		
	22.0	11.1	10.9												
T. A. Faust	25.4	12.1	13.3	8.70	6.65	23.7	11.8	12.9			25.7	11.5	14.2	[5] 7.45	[5] 5.90
	25.9	12.3	13.6	8.41	6.75						25.7	11.5	14.2	[5] 7.47	
				[5] *7.27											
				[5] *7.52											
P. F. Trowbridge	23.8	*13.9	9.9	*.45	*.57	25.7	*16.4	*9.3			28.3	*15.9	12.4	*.65	*.69
	26.4	*14.8	11.6	*.38	*.49	25.7	*15.7	*10.0			25.5	*14.0	11.5	*.51	*.50
Average soluble solids	23.5					24.3					25.2				
Average nontannins		12.0					11.4					11.9			
Average tannins			11.4					13.0					13.0		
Average glucose (A)				8.47					8.22					7.41	
Average glucose (B)					6.90					6.15					5.49
Sample No. 2.															
J. M. Seltzer	14.1	5.6	8.5	2.68	1.92	14.3	5.7	8.6	2.65	1.88	12.7	5.0	7.7	2.25	1.55
	13.9	5.7	8.2	2.64	1.83	14.7	6.0	8.7	2.67	1.90	12.3	4.9	7.4		
J. S. Rogers	13.0	4.9	8.1	2.50	1.41	17.6	5.3	12.3	2.23	1.34	13.8	4.1	9.7	2.24	
	13.7	4.9	8.8	2.21	1.30	17.8	5.1	12.7	2.13	1.46	13.5	4.2	9.3	2.07	
B. J. Ray [3]	16.6			*.00		15.0			1.89		12.7			1.83	
	14.4			*.00		15.4			1.79		13.5			1.89	
J. R. Blockey [4]	12.0	4.5	7.5	*1.55	1.39										
	12.2	4.6	7.6	*1.45	1.40										
T. A. Faust	14.0	5.0	9.0	[5] 2.10	[5] 1.50						14.9	4.7	10.2	[5] 1.95	[5] 1.27
	13.5	4.9	7.6	[5] 2.07							15.1	5.0	10.1	1.92	
P. F. Trowbridge	12.4	*7.9	*4.5	*.33	*.42	14.3	*11.2	*3.1			14.8	*6.6	8.2	*.08	Trace.
	18.6	*10.6	8.0	*.26	*.43	14.3	*10.4	*3.9			11.7	*7.1	*4.6	*.23	*.15

Average soluble solids	14.0					15.4					13.5				
Average nontannins		5.1					5.5					4.6			
Average tannins			8.3					10.5					9.0		
Average glucose (A)				2.36					2.22					2.02	
Average glucose (B)					1.53					1.64					1.41

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[1] The sugar solutions for these determinations were cleared by normal lead acetate.

[2] The sugar solutions for these determinations were cleared by basic lead acetate.

[3] The extracts used by Mr. Ray for sugar determinations stood about 2 weeks before being analyzed, and considerable fermentation had taken place, shown by growths on the surfaces of the solutions. This undoubtedly explains the results obtained.

[4] Mr. Blockey converted the cuprous oxid in the sugar determination into cupric oxid and weighed in that form. He found that the results obtained by this method checked with those obtained by the prescribed method.

[5] Sodium carbonate was used in excess instead of potassium oxalate for removing the lead.

NOTE.—Results marked with an asterisk are not included in the averages.

COMMENTS BY ANALYSTS.

B. J. RAY. (1) *Fat determination.*—Under the work of fats I have one suggestion which I believe very important. It seems to make a great deal of difference whether the material is placed in the thimble loosely or tightly. In one instance I packed the ground leather in the thimble just as tightly as I could and not split it, for our Soxhlets were rather small to hold the required 15 grams, and found to my surprise that eight hours' extraction, which I had previously found to be sufficient for the first leather, left over 4 per cent of fat behind. I then ran a series of extractions on this thimble with the results shown below. Thirty-eight hours were required to give complete extraction. So it seems to me that when work is being done on such a sample as this one, where the material may easily be packed too hard, it should be stated that the sample is to be loosely packed in the Soxhlet thimble. Where the leather was loosely packed eight hours gave complete extraction.

Time of extraction, hours:	Per cent.
8	25.19
10	29.09
12	29.16
14	29.21
16	29.25
18	29.28
20	29.30
22	29.32
24	29.34
32	29.40
38	29.43

Extraction of uncombined tannins and nontannins.—I must say that I like the alcohol method best. It is simpler, it does not require the transferring of the material from one vessel to another, and hence a chance of loss. It is shorter and easier.

Soluble solids.—Some of the solutions have stood for two weeks or more before they were used. Certain growths could be seen on the surface of the solutions. A glance at the results will show you that either the methods do not give the best results, or that some change takes place in the solutions on standing. I believe the latter. So I made up four solutions by the alcohol method on leather No. 1 and immediately determined soluble solids, after adding to Nos. 3 and 4 10 cc of chloroform. The results are shown in the following table:

Effect of standing on the determination of soluble solids.

Sample.	Soluble solids determined—		
	Immediately.	After 2 days.	After 6 days.
	Per cent.	*Per cent.*	*Per cent.*
1, no chloroform	26.57	26.18	25.53
2, no chloroform	25.17	25.00	23.52
3, 10 cc chloroform	25.60	25.31	25.28
4, 10 cc chloroform	25.51	25.27	25.00

[Mr. Ray calls attention to the fact that after 6 days' standing, in solutions No. 1 and No. 2, to which no chloroform was added, the per cent of soluble solids dropped 1.04 and 1.65 per cent, respectively, and in solutions No. 3 and 4, to which chloroform was added, the drop in soluble solids was only 0.32 and 0.51 per cent, respectively.]

I further suggest that in the evaporating of the filtrate in the determination of soluble solids, a beaker cover be used instead of a flat bottomed glass dish. Place the tared beaker covers on water bath and allow the clear solution to flow slowly in from a separatory funnel as the solution evaporates. Rinse out the funnel finally with a few cubic centimeters of distilled water. Dry in water oven to constant weight. Why dry for 12 hours? I found that in most cases only four hours were necessary for constant weight; some required six.

Glucose.—In case of sample No. 1, the results on sugar vary enormously, and I do not know how to account for this, since in two cases the duplicates agree fairly well; the other two are way off.

[Mr. Ray also states that he believes that the variations in his results on the determination of glucose are due to the long standing of the solutions before analysis. In this respect he is undoubtedly correct, for it is well known that fermentation will destroy the sugars.]

J. M. Seltzer. *Fat determination.*—The results of 6, 8, 10, and 24 hours' extraction are practically the same with leather No. 1, while with leather No. 2, 10 and 24 hours' extraction show slightly higher results over 6 and 8 hours' extraction.

Water-soluble matter.—In the Bureau of Chemistry method, when the water bath was kept at 50°, the solution in the extractor was heated to only 45°. This method shows higher results than the official A. L. C. A. method, and the resulting solution had a very much darker color, which was due to the boiling of it. I also extracted the two leathers by the A. L. C. A. method without removing the fat, and found that in sample No. 1 (following table) the results were almost the same as those in which the fat was first removed; but with No. 2 the results were very much lower by not removing the fat.

Data obtained when fat was not removed before extraction.

Method and sample.	Soluble solids.	Corrected nontannins.	Glucose A.
Sample 1:	*Per cent.*	*Per cent.*	*Per cent.*
A. L. C. A. method	23.22	12.75	8.28
Alcohol method	21.00	11.60	7.43
Sample 2:			
A. L. C. A. method	12.74	5.70	2.63

Alcohol extraction.—My results on the extraction with 95 per cent alcohol are contrary to those claimed for this method. Most of the sugar is removed by eight hours' extraction. The alcohol dissolves some matter which is insoluble in water after the alcohol is evaporated, as the water solution is very cloudy.

Sugar determination.—It has been shown before that basic lead acetate gives lower results than normal lead acetate, and my results also show the same to be true.

J. R. Blockey. *Fat determination.*—The results show that by using the prescribed method of extraction very little is to be gained by prolonging the extraction over 10 hours. Experiments were made siphoning over at about 10 times per hour, and instead of using a thimble, the leather powder was very loosely packed in the Soxhlet, in the bottom of which was placed glass wool to prevent solid particles from siphoning over. It was found that 1½ hours were sufficient to extract 29.2 per cent of the fat from No. 2, or practically as much as is extracted in 10 hours, siphoning at the rate of four times an hour and using a thimble.

On the whole it seems preferable to extract by giving 12 to 15 siphonings, and if there is reason to suppose that all of the fat has not been extracted, to continue the extraction with a fresh flask. For instance, if there is reason to suspect the presence of castor oil, then petroleum ether will only slowly extract this, as the solubility of castor oil in petroleum ether is very low. By continuing the extraction with a fresh flask the castor oil would be detected.

The drying of the fat residue until constant in weight is not always easy, as with oxidizable oils especially oxidation may take place and thereby increase the weight, and volatilization may also take place, especially with mineral oils, and thereby decrease the weight. Perfect constancy is therefore not to be expected, and it scarcely seems fair to establish set rules and time for the extraction and drying of fatty matters, as the character of the oils and fats employed varies so much.

Water-soluble matter.—A. L. C. A. method (see p. 229). Procter's extractor was employed. No difficulty was experienced in the extraction, and the liquor was coming over colorless at the end. Nontannin experiments made with American chromed hide powder gave no appreciable difference from experiments with powder chromed according to the official I. A. L. T. C. method,[1] although washing of the powder chromed with chrome alum was more difficult than with chrome chlorid, the end point with barium chlorid being not so sensitive as the end point with silver nitrate and potassium chromate.

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[1] Procter, Leather Industries: Laboratory Book on Analytical Experimental Methods, p. 212.

Alcohol method.—With regard to this method very much might be said in criticism. The first point to consider is, What do we wish to find out from the amount of soluble matter? Leather can not be considered as consisting of a definite chemical substance (composed of hide fiber and tannin) and a certain amount of excess of tan, nor can these two be quantitatively separated. There is no such sharp line of demarcation between the combined tannin and the uncombined tannin, for on washing leather there is practically no point at which the tannin ceases to be washed out. The best that can be done is an approximation, and this can be obtained quite well by the ordinary method of washing with water as exemplified in the prescribed A. L. C. A. method. For any method to supersede this method it should possess distinct advantage. One of the advantages claimed for the alcohol method over the ordinary method of extraction is that it is possible to differentiate between the natural soluble matters, such as tannins, and the artificial soluble matters, such as glucose and Epsom salts. It has been stated that 95 per cent alcohol does not dissolve glucose or Epsom salts, whereas actually it dissolves both, 95 per cent alcohol dissolving glucose to the extent of over 1 per cent. Since glucose is soluble in 95 per cent alcohol, though only to the extent of 1 per cent, the amount extracted in a Soxhlet apparatus may be noteworthy, considering the volume of alcohol which siphons through the apparatus in the course of the extraction. The results show that 6 per cent out of 8 per cent of the glucose is extracted in eight hours.

This should be sufficient to condemn the method, even if no further disadvantage could be charged against it. Another disadvantage is the increased number of operations involved and the extra time required. The evaporation of the alcohol on the steam bath is an unsatisfactory operation, as resinous substances separate out and oxidization may take place. Ninety-five per cent alcohol is expensive and according to the method can not be used over again. Moreover, if the leather is not perfectly dry before the extraction, the alcohol will no longer be 95 per cent after it has once come in contact with the leather.

The advantage claimed for this method over the water extraction method (i. e., that there is no need to estimate the mineral ash in the soluble matter as recommended by Parker and Paul) does not hold, because by both methods mineral matter is dissolved out in the extraction, so that both are liable to the same error unless the soluble mineral matter is estimated. Moreover, this determination is very easily and rapidly made.

Glucose.—The method as adopted was as prescribed, except after washing the cuprous oxid precipitate until free from alkali the crucible was removed and heated in the flame, gently at first to drive off the moisture, and strongly afterwards to convert the red cuprous oxid to black copper oxid. The crucible is allowed to cool in a desiccator and the residue weighed as cupric oxid (CuO). Preliminary experiments showed that the results were exactly the same, whether the residue was weighed as free copper or as cuprous oxid (Cu_2O) or as cupric oxid (CuO).

The precipitation of the excess of lead with solid potassium oxalate possesses one advantage over the ordinary method of using a strong solution of sodium sulphate, in that there is no dilution of the liquor, but it is doubtful if this advantage overbalances the disadvantage of the extra time taken in determining when all the lead has been removed. The same remark exactly applies to the neutralization of the hydrochloric acid by anhydrous sodium carbonate after inversion.

The ordinary method of neutralizing the acid by a known volume of strong caustic soda solution involves the dilution of the liquor and a further complication in the calculation, but it is quicker than the neutralization with the solid sodium carbonate. Experiments were performed, using the different methods, but the results showed no appreciable difference.

With regard to the difference between the use of normal and basic lead acetates it will be seen that the results in every case are higher with the normal than with the basic. It is impossible to say without a knowledge of the exact amount of glucose actually present in the leather which of the two gives the more correct results; that is, whether the figures obtained with the use of normal lead acetate are closer to the actual amount than with basic lead acetate.

Experiments were performed on solutions containing known quantities of glucose to determine, if possible, what the cause was of this higher result obtained with normal lead acetate. The results of these experiments will be published in a short time. The higher results obtained with normal lead acetate are probably due to the presence of substances like gallic acid, which reduce Fehling solution and give a precipitate with lead acetate that is soluble in dilute acids such as acetic acid. By the use of basic lead acetate some of the free acid is probably neutralized, so that the precipitation of the gallic acid and kindred substances is more complete than with normal lead acetate.

Both are liable to the same sort of error, but the error in the case of the normal is higher than with the basic, so that it seems preferable to use the latter. In any case, it is essential that the glucose determination should be performed immediately after the soluble solid extraction has been made. If the soluble solid solution is allowed to stand, decomposition takes place, and very conflicting results were obtained when experiments were made on the solutions which had been standing. By using basic lead acetate on such a solution, the amount of glucose was very much reduced, whereas in one case by using normal lead acetate the amount was actually increased. This is probably due to decomposition into substances which are not entirely removed by normal acetate, and which reduce Fehling's solution. On the whole, the use of basic lead acetate is to be preferred to normal lead acetate.

T. A. Faust. *Fat extraction.*—For extraction of grease from leather, I used a special apparatus having ground-glass joints and a Hopkins condenser, cork and rubber connections being unsatisfactory. In the case of both leathers the 24-hour extraction seemed to give abnormal results; and, in my opinion, the 8-hour extraction is sufficient for either sole, belting, or harness leather.

Extraction of uncombined tannins and nontannins (*A. L. C. A. method*).—Concordant results can be obtained by using this method, provided the temperature is always 50° and that absolutely the same temperature is kept during the extraction of different samples of leather. The method, however, is an empirical one, as a few degrees' difference in the temperature makes a considerable difference in the results. There is a constant breaking down of leather substance by the continued digestion with water even at this temperature, thus making the results depend entirely upon the temperature, time of extraction, and degree of tannage of the leather. The results by this method are not as concordant as those by the alcohol method.

Bureau of Chemistry method.—The apparatus as suggested in the Bureau of Chemistry method is rather cumbersome, and considerable difficulty in manipulation was experienced. Constant attention was required to prevent the temperature in the water jacket from getting above 50°, and it was very difficult to drive the steam through the water jacket at this temperature because the steam was condensed. It was almost 2 hours before the first siphoning took place. The result on leather No. 1 is lower than by either the alcohol or the A. L. C. A. method of extraction, and I believe it is due to the fact that the apparatus did not siphon often enough. I have no results on leather No. 2, as I was not favorably impressed with the method and did no more work with this apparatus.

Alcohol extraction.—This method seems to be very promising, as it is not empirical to the extent that the water extraction is, where no definite end point is reached, nor is there the breaking down of leather substance and dissociation of tannin that there is in the water extraction. The tannin extracted by alcohol is, I believe, actual uncombined tannin, which is more than I can say of the tannin extracted by water. An examination of the leather residues after extraction with water showed a more or less dissociated and jumbled mass of leather, whereas in the alcohol extraction the leather seemed to retain practically its original condition. It is true that Epsom salts are soluble in alcohol, but to a limited extent. I believe that this error can be ignored for commercial analyses.

Glucose, however, is quite soluble in alcohol, so that there is probably no advantage in the alcohol method in this respect.

Glucose.—I think that basic lead acetate gives too low results and consequently did not complete all determinations with this reagent. Sodium carbonate was used in this work instead of potassium oxalate, as two samples which I have tried with potassium oxalate ran 8.70 and 8.41 per cent of glucose, as against 7.27 and 7.52 per cent (as reported) using sodium carbonate. This led me to believe that the results with potassium oxalate were too high, and I fell back on the sodium carbonate, which I have always used in the past. I believe, however, that potassium oxalate, being a neutral salt, is preferable to sodium carbonate, as it is quite difficult to know just when enough sodium carbonate is added to precipitate all of the lead. In my first report I mentioned the fact that difficulty was experienced in securing good duplicates, and I have no doubt by this time that this was caused by the fact that in adding an uneven amount of sodium carbonate in order to be certain of the removal of all lead, conditions of alkalinity were established in the filtrate which caused the poor duplicates. Excellent duplicates were secured on the actual precipitation of the glucose in the filtrate after inversion and neutralization, proving that the source of my trouble was not in the handling of the copper precipitate.

I think the method which you have outlined for the work on the subject this year is much better than the present method. I believe the refluxing to be a great improvement over the evaporating, and I also believe your proportion of lead acetate to the volume of solution to be better than that called for by the present method. Personally, I would prefer taking a little larger quantity of the solution, which would obviate the necessity of waiting so long for sufficient filtrate from which to remove the lead and yet have the 150 cc required for inversion. The determinations of glucose in water extract and alcohol extract were fairly concordant.

GENERAL SUMMARY BY REFEREE.

DETERMINATION OF FATS.

The results on the extraction of fats taken as a whole agree very well. In sample No. 1, which contained about 2 per cent fat, judging from the averages as shown in Table 1, all except 0.03 per cent of the fat was removed in 6 hours. In sample No. 2, containing from 29 to 30 per cent of fat, the averages indicate that 10 hours' extraction removes all but 0.08 per cent of the fat present. Table 1 shows that in the results obtained by the 24 hours' extraction both in samples No. 1 and No. 2 there are a few abnormally low results. These, I think, may be due (as suggested by Ray and Blockey) to the leather being packed too tightly in the extraction thimble. No mention was made of this precaution in the directions, because it was thought that the necessity of loosely packing the leather in the Soxhlet was self-evident. If the packing of the leather be carefully avoided and the siphonings regulated to approximately 10 per hour, I believe that 10 hours' extraction will be more than sufficient to completely remove the fat, even from a heavily greased leather, and that leathers containing a low percentage of fat can be extracted in from 6 to 8 hours.

EXTRACTION OF SOLUBLES.

After comparing the three methods of extraction, I give the Bureau of Chemistry method the preference. Some claim that this method is cumbersome and the apparatus difficult to arrange and manipulate. I have found no trouble in this direction; in fact, it requires only a small amount of ingenuity to arrange and manipulate the apparatus as described in the directions. Of course it is not expected that the side tube of the Soxhlet will pass through the water bath which surrounds the extractor and which is kept at 50° C. This tube must pass to the condenser outside of the bath or there will be a large amount of condensation and running back into the extraction flask and the extraction will progress very slowly. Although the total time required for the extraction is longer than that in either of the other methods, the time actually used in manipulation is but little more. The results for soluble solids and tannins are higher than by the A. L. C. A. method, while the nontannins remain practically the same. This indicates that while a small amount of tannin may be destroyed by boiling the extract, more of the uncombined tannin present is removed. The A. L. C. A. method undoubtedly removes practically all of the readily soluble tannins, but the more difficultly soluble tannins, such as the "reds," require much longer extraction, such as is given by the Bureau of Chemistry method. Since the extracts obtained by this method, when tested for soluble hide, gave no indication of its presence, it is evident that practically no combined tannin was removed; and since the soluble solids obtained by the Bureau of Chemistry method are usually about the same or a little lower than those by the alcohol method, it seems that Mr. Faust's comment, that extraction by the Bureau of Chemistry method removes combined tannin and breaks up the leather, is not well-grounded. The A. L. C. A. method gives very satisfactory results, save for the fact that it does not remove the more difficultly soluble tannins.

The alcohol method, contrary to the claims of Riker, does remove nearly all of the sugar present. (See Table 2, Glucose B.) The extract, when evaporated with water, always becomes cloudy, and considerable gummy, resinous matter remains insoluble, making manipulation difficult. Sometimes it is nearly impossible to obtain a clear soluble solid filtrate. In my own experience, I have found the results by this method little higher than those of the Bureau of Chemistry method, and I do not find a sharper end point in the extraction. A leather residue, after being extracted with vigorously boiling 95 per cent alcohol for 8 hours, was washed once with water, and the washing water gave a heavy precipitate with a gelatin salt solution.

GLUCOSE.

A comparison of the results obtained for Glucose A (cleared with normal lead acetate) and Glucose B (cleared with basic lead acetate) is very interesting. Sample No. 1 (Table 2) was a specially prepared leather and contained a definitely known amount of glucose. The amount actually added was 7.73 per cent, and the blank on the original leather gave 0.60 per cent, making 8.33 per cent total sugar in the leather. It is evident from Table 2 that in every case where basic lead acetate was used as a clearing agent the percentage of sugar found was much lower than the percentage of sugar actually added. Furthermore, the average of all sugar determinations made on extracts obtained by the A. L. C. A. and the Bureau of Chemistry methods and cleared by normal lead acetate was 8.39 per cent, or within 0.06 per cent of the theoretical amount of sugar present. In sample No. 2 (Table 2) the results for Glucose A and Glucose B indicate that the same rule holds good. This shows clearly that theoretical results can be obtained with normal lead acetate and that the basic lead acetate removes sugar and invariably gives low results.

RECOMMENDATIONS.

It is recommended that the following methods for the analysis of leather be adopted as provisional methods:

1. PREPARATION OF SAMPLE.

Reduce the sample to state of fineness such that it will pass through a millimeter sieve (it must not contain hard lumps). Avoid all unnecessary heating during grinding. Spread the sample out and allow it to return to atmospheric moisture conditions, mix thoroughly, and place in a tightly covered container.

2. MOISTURE.

Weigh 10 grams of the prepared sample into a wide, shallow, covered weighing bottle (or a similar dish which may be covered tightly when it is removed from the drying oven), and dry for 15 hours in a water oven at from 98° to 100° C. Cover the weighing bottle when it is removed from the oven, desiccate over sulphuric acid, and weigh. If it is desired to determine the moisture originally present, the leather must be quickly cut in small pieces with a knife and dried in the usual way without grinding.

3. ASH.

Slowly incinerate 5 grams of the prepared sample at a dull-red heat. (In case difficulty is experienced in burning off the carbon, leach out the residue with hot water, filter on an ashless filter, dry and ignite the filter and residue, add the filtrate, evaporate to dryness, and ignite.) Desiccate over sulphuric acid and weigh.

4. FATS.

Place loosely 15 grams of the prepared leather in a Soxhlet extractor containing a layer of fat-free cotton and cover with another layer of cotton. Extract with petroleum ether, distilling between 50° and 80° C. for from 8 to 10 hours. Heavily greased leathers (containing 15 per cent or more) will require the maximum time. Regulate the boiling so as to give from 10 to 15 siphonings per hour. Remove the flask, evaporate the petroleum ether on a steam bath, and dry in a water oven at from 98° to 100° C. to practically constant weight. Desiccate and weigh. Retain the leather residue from the fat extraction for the extraction of water solubles.

5. EXTRACTION OF WATER SOLUBLES.

Thoroughly moisten the leather residue from the fat extraction. Transfer to a Soxhlet extractor designed for making extractions at low temperatures and containing a layer of cotton. Place cotton above the leather in the extractor to prevent it from being carried over at the time of siphoning and extract at 50° C. At the beginning of the extraction, pour 25 cc of distilled water (including that used in moistening the leather) into the Soxhlet and allow it to siphon into the flask below, then begin the boiling. At the end of the first hour, remove the flame and transfer the extract to a 1-liter graduated flask. Add 175 cc of distilled water to the Soxhlet and continue the extraction for 2 hours. Transfer the extract to the graduated flask, add 175 cc water, and extract for 3 hours. Transfer the extract to the graduated flask, add 175 cc water and extract for 4 hours. Transfer the last portion of the extract to the graduated flask. This gives 14 hours' extraction and an extract that does not exceed 1 liter in volume. Make the extract up to 1 liter at room temperature. A few drops of toluene or carbolic acid must be added to the leather extract to prevent the fermentation of sugars.

6. GLUCOSE.

(a) *Preparation of solution.*

To 200 cc of the leather extract add 25 cc of a saturated solution of normal lead acetate, mix thoroughly, allow to stand about 15 minutes, and filter. The funnels and beakers must be kept covered to prevent evaporation. Precipitate the lead from this filtrate with a slight excess of solid potassium oxalate and filter. Pipette 150 cc of the last filtrate into a 600 cc Erlenmeyer flask, add 5 cc of concentrated hydrochloric acid and boil under a reflux condenser for two hours. Cool, neutralize with anhydrous sodium carbonate (using phenolphthalein), transfer to a 200 cc graduated flask, and make to volume. Filter through a double filter. (This filtrate must be clear.)

(b) *Determination.*

Use 50 cc of this filtrate for the determination of sugar, following the method for determining sugars in general (Munson and Walker), Bulletin 107, Revised, pages 241–242.

7. TOTAL SOLIDS.

Thoroughly mix the original extract, immediately pipette 10 cc into a tared dish, evaporate, and dry as directed in "Methods for the analysis of tanning materials," Bulletin 107, Revised, page 37, under "4. Evaporation and drying."

8. SOLUBLE SOLIDS.

Determine as directed under "Methods for the analysis of tanning materials," Bulletin 107, Revised, page 36, "(c) Soluble solids."

9. NONTANNINS.

Prepare a quantity of hide powder sufficient for the number of analyses to be made *during one day only*, in the following manner: Digest with 10 times its weight of water until thoroughly soaked. Add 3 per cent of chrome alum in solution, agitate by either shaking or stirring occasionally for several hours, and let stand overnight. Wash by squeezing through linen, continuing the washing until the wash water gives no precipitate with barium chlorid. Squeeze the hide, using the press if necessary, so that the wet hide will contain between 70 and 75 per cent of water. Use approximately 20 grams of wet hide for moisture determination. Add to 200 cc of the original solution such a quantity of wet hide as will give the ratio of dry hide to tannin indicated by the following table:

Tannin range per 100 cc.	Dry hide powder per 200 cc.
0.35 to 0.45 gram	9 to 11 grams.
.25 to .35 gram	6.5 to 9 grams.
.18 to .28 gram	4 to 6.5 grams.
.00 to .15 gram	0 to 4 grams.

Add 2 grams of kaolin to the filtrate, stir, and filter through a folded filter S and S No. 590 of sufficient size to hold the whole filtrate, returning until clear. Evaporate 100 cc of the filtrate, dry, and weigh as directed under soluble solids. The weight of the residue must be corrected for the dilution caused by the water contained in the wet hide powder. (The nontannin filtrate must not give a precipitate with a 1 per cent gelatin, 10 per cent salt, solution.)

NOTE.—In order to limit the amount of air-dried powder used, determine the moisture in the air-dried hide powder and calculate the quantity equal to at least 15 grams of actual dry hide powder, take any multiple of this quantity, according to the number of analyses to be made, and after chroming and washing as directed, squeeze to a weight representing 70 to 75 per cent water. Weigh the whole amount and divide by the multiple of the grams of actual dry hide powder taken to obtain the weight of the wet hide powder for 200 cc of solution. *The hide powder should always be used the same day that it is washed.*

10. TANNIN.

The tannin content is shown by the difference between the soluble solids and the corrected nontannins.

11. NITROGEN.

Determine by the Kjeldahl or Gunning method, Bulletin 107, Revised, pages 5–7.

12. HIDE SUBSTANCE.

Multiply the percentage of nitrogen by 5.62 and the result will be the percentage of hide substance present.

It is further recommended that the work be continued, paying special attention to the development of tests indicative of the quality and durability of leather.

Mr. Mitchell, referee on food adulteration, moved that another associate refereeship on this subject be established to cover work on the determination of heavy metals in food products.

The motion was carried.

The remainder of the session was devoted to the work on drugs and medicinal plants, the following reports of referees and correlated papers being presented in abstract or by title.

REPORT ON MEDICINAL PLANTS AND DRUGS.

By L. F. Kebler, *Referee.*

The work during the past year has been of singular interest in many ways, but the amount accomplished is not as much as desired, chiefly because of lack of available workers. The results again emphasize the great need of systematic study, sampling, analytical methods, and standards, both specific and applied, with a view to determining what degree of accuracy can be obtained by workers in the same and different laboratories.

The Pharmacopœia prescribes tests for all drugs recognized by this authority, including the source, analytical methods, standards, degree of purity, and limit of foreign matter, as the case may be. The subject of sampling is receiving careful consideration, and it is hoped that a full report with recommendations can be made at the next meeting. The source included in some of the tests is frequently of the utmost importance; for example, cardamon, "The dried, nearly ripe fruit of *Elettaria repens* (Sonnerat) Baillon (Fam. Zingiberaceæ)." This standard clearly designates the source of the material, and if a sample should be found which is contaminated with bastard cardamon it is clearly evident that the article is not the article prescribed by the law. Many of the tests for crude drugs make no provision whatever for the presence of certain incidental or accidental foreign material that is usually present in these products.

Uva ursi has been offered for importation containing an excess of 40 per cent of stems, worthless leaves, and other foreign material. The excuse offered by the importer for the character of the drug was that it is intended for use in the manufacture of cattle powder or veterinary remedies. Steps have been taken to reduce the percentage of such material imported; in fact, certain imports have been permitted entry only on condition that the importer would eliminate the excessive amounts of foreign material.

An excellent example for this recommendation to be put into practice is in cubeb berries. There is no legal excuse for cubeb berries containing more than 5 per cent of foreign material, since, as commonly found in this commodity, it can be easily eliminated by mechanical means.

Experience has furthermore shown that it will be necessary to prohibit the importation of commodities containing excessive amounts of foreign material if the pharmaceutical and medical professions are to be supplied with agents suitable for treating the sick to the best advantage. It is hoped that prime goods will in time be offered free from impurities; in fact, the courts may make this necessary in order that the public may be protected. In the past certain goods have been released on the condition that they be marked indicating the nature and character of the impurities present. It subsequently developed that some of these goods were supplied on orders without indicating to the purchaser that the articles were not of proper quality. Such transactions at times result in the second dealer finding himself face to face with a violation of the law. Furthermore, such goods are at times shipped into interstate commerce with the correct marks, but after they have crossed the borders of the State the original package is broken and the consuming public is at the mercy of the dealers unless State officers interfere. It is hoped, therefore, that the officers charged with the supervision and enforcement of the State drug laws will take an active interest in these matters.

Some of the analytical methods did not give satisfactorily concordant results in the hands of different workers. For example, the method for determining the alkaloidal matter in Hyoscyamus is liable to a variation of 100 per cent when executed by experienced analysts, and naturally a method giving such great variations as this would be difficult to employ to advantage for court work. The chief drawback with the method appears to be insufficient time and solvent to extract the alkaloids properly from the powdered drug.

Specific standards are prescribed in the tests for certain drugs. For example, the description of *Cannabis indica* states that the powder shall contain few or no stone cells, which means the virtual absence of seeds in the finished product. In practice it has been found exceedingly difficult to put such a standard in force and effect for the simple reason that there are few, if any, samples available on the market free from seeds to the extent prescribed. Furthermore, information is lacking as to just what amount of seed should or should not be permitted.

Attention is now called to standards that are indicated but not specifically set forth. Such standards include the amount of alcohol that should be present in finished products made according to certain prescribed formulas. For example, the Pharmacopœia gives a formula for manufacturing tincture of ginger, but no definite statement appears as to the amount of alcohol that should be present in the finished product, neither is there any reference as to the amount of nonvolatile matter that might possibly be present in a preparation made as directed by this authority. These features are most important, because it is well known that the alcoholic strength of the menstruum is quite different from the percentage of alcohol contained in the finished product. Ninety-five per cent alcohol usually extracts far less material than this solvent containing more water. This feature is taken advantage of by some manufacturers in preparing such articles as tincture of ginger or extract of ginger.

In a paper communicated to this association last year by Mr. Street on the subject of ginger extracts, attention was called to the fact that menstrua containing smaller amounts of alcohol than that contained in original (95 per cent) alcohol removed considerably more extractive matter than this 95 per cent alcohol. Your referee has conducted experiments on the same article with results that corroborate the findings of Mr. Street in this particular. The results will be communicated next year in a separate article entitled "Standard for tincture of ginger." The work was completed this year, but owing to other duties and absence from the city the results were not presented.

In this connection it might be well to call attention to the well-known fact that examinations and investigations of food and drug problems frequently overlap each other, and if it were possible to arrive at some plan whereby the efforts of investigators on subjects that have to a certain extent a common basis might be combined, it certainly would be most desirable. Such a plan would eliminate so-called double standards in many instances.

The associate referee on synthetic products, W. O. Emery, has conducted cooperative work during the past year and the report will be submitted.

H. C. Fuller has also conducted some cooperative work on medicated soft drinks and the report is presented.

Mr. Schneider has submitted a very voluminous paper on the subject of microscopical work, but the referee feels that a part of this paper does not deal specifically with the work assigned him as associate referee, and it is therefore suggested that only such parts of his report be considered as properly come within the scope of the work on medicinal plants.

Several papers on various subjects are presented. In some instances the results embodied in these papers involved cooperation, while others represent the work of a single individual.

In concluding, the referee feels that there has not been sufficient work done along the various lines to make definite recommendations, and for this reason simply recommends that the work at present established be continued, with such modifications in the methods as the several associate referees have recommended.

REPORT ON HEADACHE MIXTURES.

By W. O. Emery, *Associate Referee, Synthetic Drug Products.*

Cooperative work on headache preparations has been conducted during the past year according to the general plan put in operation four years ago and reported on at the last three meetings of the association. Two samples were submitted to cooperating chemists, one (No. 8) a powder mixture containing acetphenetidin, caffein, and sodium bicarbonate; the other (No. 9) a liquid product consisting of alcohol, acetanilid, caffein, sodium salicylate, water, glycerin, and oil of peppermint, of which constituents the first four named were to be estimated quantitatively. No. 8 was to be treated substantially in accordance with instructions outlined in Bulletin 132, page 198; for No. 9 the following directions were sent to the co-workers:

METHOD FOR ANALYZING LIQUID HEADACHE MIXTURE.[1]

ALCOHOL.

Cool sample to 25° C., at which temperature fill a 25 cc flask[2] to mark, weigh, then transfer to a distilling apparatus,[3] rinsing out the flask several times with water, employing for this purpose a total of 25 cc. Distil into a 50 cc flask (surrounded by ice water) until the liquid in the distilling flask is reduced to 10 cc. Transfer the somewhat turbid distillate (due to volatile oil) to a separator provided with short delivery tube, rinse out the flask several times with a total of 15 cc water, saturate the combined solution with sodium chlorid, shake vigorously for two minutes with 25 cc low boiling (40–60° C.) petroleum ether, then allow to stand 15 minutes. Draw off entire liquid into a second separator, washing out the first (which still contains undissolved salt) with 5 cc saturated salt solution into the second. Draw off the lower layer into first separator, from which the undissolved salt has in the meantime been removed, and shake it out as above with a second portion of 25 cc petroleum ether. Combine both ethereal extractions, wash with 25 cc saturated salt solution, draw off latter after 15 minutes and add to the distillate first treated, finally transferring resultant mixture to the distillation flask.[4] Distil into a 50 cc flask (cooled in ice water) nearly to mark, allow temperature to rise to 25° C. fill to mark, and determine the specific gravity at this temperature in a Squibb pyknometer, calculating the per cent of alcohol by volume from tables in the United States Pharmacopœia, eighth revision. The percentage obtained multiplied by 2 will give the amount of alcohol in original sample.

CAFFEIN-ACETANILID.

Transfer residual crystalline magma containing the caffein, acetanilid, and sodium salicylate from distilling flask to a separatory funnel by the aid of successive portions of water (ending with a little chloroform if necessary to remove all traces of acetanilid) so that the final volume of the aqueous solution does not exceed 50 cc. Make five separate extractions by means of vigorous shaking, each time with 70 cc chloroform. Allow solvent to clear after each extraction, pass through small (5.5 cc) dry filter into a 200 cc Erlenmeyer and distil by the aid of a small flame until about 60 cc have gone over. Use this distillate for each subsequent extraction, making up to 70 cc as found necessary with fresh solvent. After the final extraction and distillation of chloroform, add 10 cc dilute sulphuric acid and evaporate to 4 or 5 cc on steam bath, transfer the residual acid solution of caffein and anilin sulphate to a 100 cc graduated flask, fill to mark and determine caffein and acetanilid in an aliquot of 25 cc, as directed in method previously submitted. (See Bul. 132, p. 197.)

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[1] Sample was prepared at the temperature of 25° C. in a flask graduated at 20° C. since it was found that at the latter temperature the acetanilid tended to separate in part from the solution.

[2] Only flasks graduated at 20° C. should be employed in determining the alcohol, during manipulation with the sample in hand, for reasons which are apparent after a consideration of the footnote above.

[3] The apparatus consists of a half-liter, round-bottom flask standing on a perforated (1-inch) asbestos plate, so that the flame may never come in actual contact with any portion of the glass not covered by the liquid contents. In this way overheating may easily be avoided. The connection between flask and condenser is effected by means of tightly fitting rubber stoppers and doubly bent tube. The lower end of condenser is contracted sufficiently to permit entrance into neck of receiving flask immersed in ice water, thus avoiding use of adapter.

[4] This method for the removal of volatile oils is substantially the same as that suggested by Thorpe and Holmes, J. Chem. Soc., 1903, 83: 314.

SODIUM SALICYLATE (SALICYLIC ACID).

The aqueous solution remaining in separatory funnel and containing the sodium salicylate and glycerin is rendered acid with hydrochloric acid and extracted three separate times by means of vigorous shaking, 70 cc chloroform being employed for each extraction. The solvent, after clearing, is run into a 200 cc erlenmeyer (containing 10 cc water and 1 gram dry sodium carbonate, sufficient to fix all the salicylic acid present) and distilled over a small flame until most of the chloroform has been expelled. After the final distillation and elimination of all chloroform, transfer the aqueous soda solution of sodium salicylate to a liter flask, add 10 grams dry sodium carbonate, fill to mark, pipette an aliquot of 100 cc into a 200 cc erlenmeyer, heat nearly to boiling, then add about 35 cc fifth normal iodin in potassium iodid (or double this quantity of tenth normal iodin solution), enough to insure an excess of iodin. Heat one hour on steam bath to near the boiling temperature, during which time a violet-red precipitate of tetraiodophenylenquinon $(C_6H_2I_2O)_2$ will appear. Remove excess of iodin by the addition of a few drops of hypo solution, decant liquid off into a tared Gooch crucible, care being taken that most of the precipitate remains in the flask. Add 50 cc boiling water to the iodin compound in flask, digest for 10 minutes on steam bath, then pour into the gooch, into which the precipitate is gradually washed by means of hot water, using for this purpose about 200 cc. Dry to constant weight in air bath at 100° C. Multiply weight of precipitate by 0.4658 and the product will be the weight of sodium salicylate in aliquot taken. Report all results in parts per 100.

ANALYTICAL DATA.

The results obtained and reported have been tabulated as follows:

Cooperative results on headache mixtures.

[Percentage data.]

Analyst.	No. 8.				No. 9.			
	Chloroform-insoluble residue.	Caffein.	Acetphenetidin.	Total.	Alcohol.	Caffein.	Acetanilid.	Sodium salicylate.
J. M. Bartlett, Orono, Me	20.66	12.30	64.50	[1] 99.30	29.50	0.47	3.38	5.20
	19.63	13.80	67.60					
		13.50	66.10					
		13.00						
W. O. Emery, Washington, D. C.	21.10	13.12	65.67	99.89	29.34	.44	3.49	5.22
					29.50	.44	3.48	5.21
Chas. W. Johnson, Seattle, Wash.[2]	20.88	12.61	66.59	100.08	29.36	.45	3.48	5.26
	20.92	11.91	66.27	99.10				
C. C. Le Febvre, Washington, D. C	20.92	13.17	66.27	100.36	29.50	.43	3.46	5.16
	20.96	13.46	65.56	99.98				5.17
H. M. Loomis, Seattle, Wash	[3] 21.78	13.02	66.76	101.56	29.26	.48	3.38	5.12
	[3] 21.19	13.62	66.84	101.65		.48	3.38	5.15
C. B. Morrison, New Haven, Conn.[4]	[3] 20.86	13.30	66.60	100.76	29.67	.44	3.51	5.26
		13.30	66.30		29.50			

[1] Total of averages.
[2] No. 8 done by Josephine Johnson. No. 9 by J. J. Wi tler.
[3] Result of titration.
[4] Reported by J. P. Street.

Cooperative results on headache mixtures—Continued.

Analyst.	No. 8.				No. 9.			
	Chloroform-insoluble residue.	Caffein.	Acetphenetidin.	Total.	Alcohol.	Caffein.	Acetanilid.	Sodium salicylate.
R. R. Shively, Washington, D. C.	20.71	13.52	65.61	99.84	29.00	0.45	3.48	5.31
	21.07	13.24	65.73	100.04		.46		5.29
H. L. Schultz, Detroit, Mich.....	20.70	12.85	66.40	99.95		.48	3.44	5.20
	20.80	12.30	66.30	99.40		.47	3.44	5.29
	20.66	13.40	65.60	99.66				
Average....................	20.86	13.08	66.16	100.11	29.40	.46	3.45	5.22
Maximum........................	21.78	13.80	67.60	....	29.67	.48	3.49	5.31
Minimum........................	19.63	11.91	64.50	....	29.00	.43	3.38	5.12
Difference........................	2.15	1.89	3.10	....	.67	.05	.11	.19
Known composition [1]............	20.83	12.50	66.67	....	30.00	.45	3.56	5.36

[1] No. 8 prepared by mixing in mortar and repeated sifting of the air-dried ingredients in the proportion of: Acetphenetidin 80, sodium bicarbonate 25, caffein 15. No. 9 prepared by dissolving acetanilid 70.1 grams, caffein 8.78 grams, sodium salicylate 105.15 grams, oil peppermint 20 drops, glycerin 10 cc in 526.3 cc alcohol (95 per cent), and sufficient water to make 2 liters at 25° C. Such a mixture was calculated to yield a preparation containing alcohol 30 per cent, and acetanilid 16 grains, caffein 2 grains, sodium salicylate 24 grains per fluid ounce.

DISCUSSION OF RESULTS.

Mr. Le Febvre reports observing on various occasions a whitish crystalline sublimation on the sides of dish used in heating the acetphenetidin residue at the full temperature of steam bath, in order to expel any excess of acetic anhydrid. This behavior indicates volatility of the former substance when heated unduly or for a long period, and probably explains the low values obtained by used a larger (300 cc) flask in his operations.

Mr. Loomis finds great difficulty in distilling chloroform from a 100 cc Erlenmeyer as specified in the directions, since it tends to suddenly boil up violently, some of the solution passing over into the condenser tube. He accordingly used a larger (300 cc) flask in his operations.

Mr. Street reports, in connection with the determination of sodium salicylate, that in an experiment with 0.1000 gram pure salicylic acid he obtained 0.2500 gram of the red iodin compound, or, using the factor 0.4012, an equivalent of 0.1003 gram of salicylic acid.

The tabulated results on No. 8 show a considerable range of variation, sufficient, in fact, to warrant further investigation with the same or a similar combination. These variations are due in part, doubtless, to inexperience; partly, also, to imperfections in the method itself, the exact nature of which does not at this time appear entirely clear. It has been known for some time that acetanilid, when heated above 40° C. in the dry state, suffers appreciable loss through sublimation; accordingly, it was found advantageous in estimating this substance to adopt a volumetric method. On the other hand, it was believed until quite recently that acetphenetidin could be heated for a long time at the ordinary temperature of the steam or vapor bath without material loss. It appears, however, that greater care will have to be exercised in all operations calculated to free acetphenetidin from the last traces of acetic acid and anhydrid. Aside from the apparent necessity of avoiding any undue excess of the anhydrid used in the acetylating process, it is suggested that only moderate heat be applied after each addition of the chloroform-alcohol mixture. Thus, after expelling the major portion of acetic anhydrid on a moderately heated steam or vapor bath, the last traces will be more safely removed by permitting the evaporating dish to stand 24 to 48 hours in the open prior to weighing, or until repeated weighings indicate no further loss.

The objection to the employment of a 100-cc Erlenmeyer for the distillation of chloroform containing a dissolved substance as caffein or acetanilid is undoubtedly well taken, though any violent ebullition experienced by Mr. Loomis, and possibly, also, by others, can usually be avoided by gentle heat together with the presence of one or more glass beads in the distillation flask. However, a larger flask presents decided advantages, in that the rather numerous distillations required can be effected with greater expedition and without fear that any of the dissolved substances may be carried over mechanically with the chloroform vapor. At present a 200-cc Erlenmeyer is used in the Bureau of Chemistry, in connection with a spray trap substantially like the accompanying illustration (fig. 3).

A further change in the procedure outlined under caffein has been found advantageous. Instead of two extractions with chloroform of 80 cc each, we have gone over definitely to the alternative of three extractions of 60 cc solvent, substantially as given in footnote (*c*), page 197, Bulletin 132. Suitable recommendations covering the points involved have been presented to the association.

The values obtained on No. 9 are reasonably close in agreement, particularly so in the case of alcohol, when one considers the opportunity for loss presented in the operation to eliminate essential oil from the alcoholic distillate. The flask and condenser used in determining the alcohol are shown in their relative dimensions in figure 4.

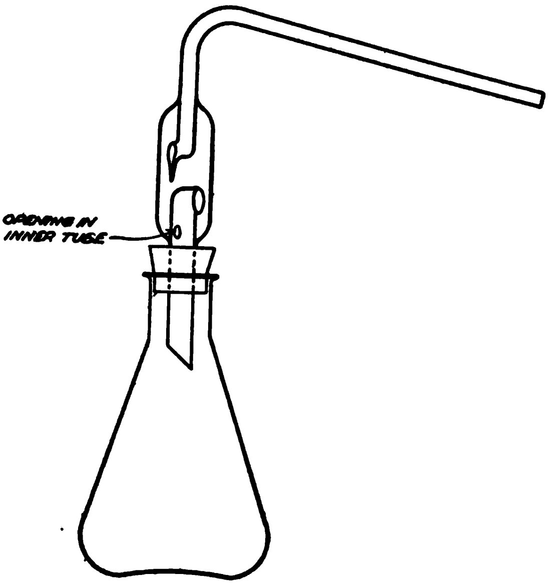

FIG. 3.—Spray trap.

During the course of our investigations of headache and other medicinal preparations new problems constantly arise, involving, in whole or part, the methods already presented to this association. More frequently, however, it is found necessary to modify or amplify these methods so as to fit the particular combination under investigation. Two such problems I desire to bring to your attention at this time, namely, the treatment of mixtures containing, on the one hand, codein, caffein, and acetphenetidin; on the other, antipyrin and acetanilid. The method followed in the first case is substantially as follows:

CODEIN, CAFFEIN, AND ACETPHENETIDIN MIXTURE.

Codein.

Weigh out about 0.3500 grams of powdered material (if in pill or tablet form at least 10 of these should be reduced to powder), transfer to a separatory funnel by means of 10 cc very dilute sulphuric acid (or sufficient to render the solution decidedly acid after neutralization of any carbonate that may be present), extract by means of vigorous shaking with 50 cc of chloroform. After clearing draw off the solvent, allowing it to run through a small (5.5 cm) filter into a 200 cc erlenmeyer. Distil off about 50 cc chloroform, using a small Bunsen flame. Extract a second and third time with same amount of solvent as first used. Allow the chloroform from each extraction to run into the erlenmeyer, then distil off all but about 10 cc. Now add 10 cc dilute sulphuric acid (1 volume concentrated acid to 5 of water) and heat on steam bath until the chloroform has disappeared and only about 5 cc of the acid liquid remains, then treat as directed under caffein.

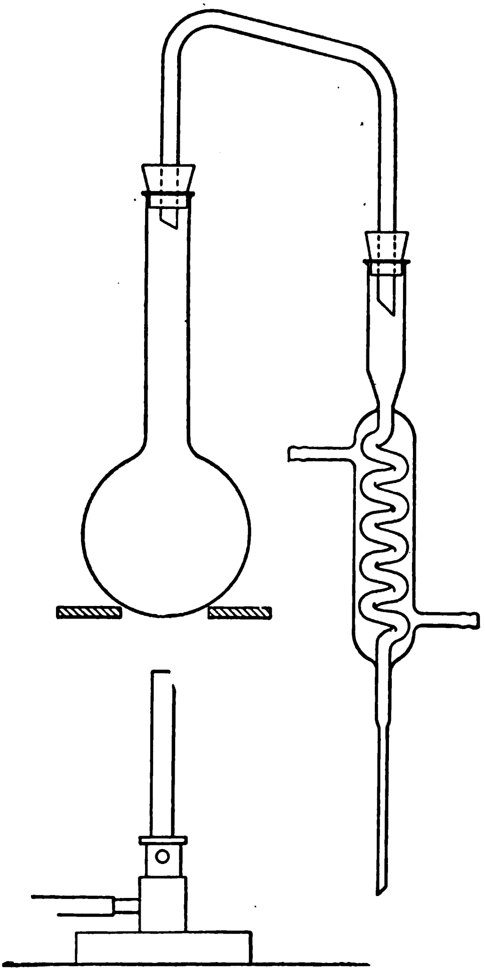

Fig. 4.—Alcohol distillation apparatus.

Render the acid liquid in separator containing the codein sulphate neutral by the addition of solid sodium bicarbonate, wash out filter used in the preceding operation to clarify the chloroform, once with water, allowing latter to run into the separator, then reextract three separate times with 50 cc chloroform. Collect solvent as above directed in a second 200 cc erlenmeyer, distilling off most of the liquid by the aid of gentle heat. Transfer residual chloroform to a small beaker or evaporating dish, using sufficient fresh chloroform for this purpose, heat gently over steam bath to dryness, cool, and weigh as anhydrous codein.

Caffein and acetphenetidin.

The aqueous acid solution containing the caffein and phenetidin sulphate is transferred to a separatory funnel and treated substantially as directed under the respective headings on page 198, Bulletin 132.

The procedure covering the second case is as follows:

ANTIPYRIN AND ACETANILID MIXTURE.

Antipyrin.

If in powder form (pills and tablets should naturally be reduced to such condition), weigh out 0.2 to 0.4 gram in a 50 cc erlenmeyer, add 10 cc dilute sulphuric acid (1 volume concentrated acid to 5 of water) and heat on steam bath till the residual liquid amounts to about 5 cc. Pour and rinse into a separatory funnel so that final volume does not exceed 20 cc. Extract 5 times with chloroform, using 50 cc for each extraction. Pass the solvent through a small (5.5 cm) dry filter into a 200 cc erlenmeyer and distil over a small flame down to about 10 cc. Tranfer the combined residue obtained after the fifth extraction by means of small quantities of chloroform to a small tared beaker or crystallizing dish, evaporate solvent at moderate heat and at the ordinary temperature, dry the residual antipyrin in desiccator to constant weight.

Acetanilid.

Transfer the acid solution remaining in separator and containing the anilin sulphate to a 100 cc erlenmeyer, heat on steam bath to expel traces of chloroform. Wash out the filter used in the preceding operation to clarify the solvent, once with water, allowing the latter to run into solution of anilin sulphate. Add 10 cc concentrated hydrochloric acid, a like amount of water, titrate with a standard solution of potassium bromid-bromate, then proceed substantially as directed on page 198 under the respective heading in Bulletin 132.

In the event that the mixture of acetanilid and antipyrin contains in addition caffein, sodium bicarbonate, starch, or sugar, a powdered sample should be weighed out on a tared filter and chloroform used to separate the first three mentioned ingredients. After treating such a mixture of acetanilid, antipyrin, caffein, etc., with dilute sulphuric acid on steam bath and subsequently with chloroform, the antipyrin and caffein would be obtained together and can later be separated by means of mercuric nitrate.

Mixtures of antipyrin and acetphenetidin are treated in a manner similar to that for the separation of antipyrin and acetanilid up to the point of obtaining phenetidin sulphate. The acetphenetidin is then regenerated and weighed as such, according to directions given for the analysis of acetphenetidin headache mixtures.

REPORT ON MEDICATED SOFT DRINKS.

By H. C. Fuller, *Associate Referee.*

Ten collaborators reported results on the determination of solids, caffein, cocain, and phosphoric acid in a soft-drink sample, using the methods as given in Bulletin 137, page 190, or Circular 66, page 7.

The results were obtained on a mixture prepared according to the following formula:

	Grams.
Water	10,000
Sugar	10,000
Caffein	50
	cc.
Fluid extract coca	200
Phosphoric acid	100
Lime juice	100
Caramel	100
Flavoring, consisting of the following	200
Oil of coriander	8
Oil nutmeg	15
Oil cassia	30
Alcohol	200

The following results were reported:

Results reported by collaborating chemists.

Analyst.	Solids.	Caffein.	Cocain.	Phosphoric acid.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
M. C. Albrech, Pittsburgh, Pa	48.08	0.23	0.002	0.53
Edmund Clark, Boston, Mass	44.30	.20	.002	.45
	44.77	....	....	.48
E. O. Eaton, Washington, D. C	54.1	.23	.002	.53
W. O. Emery, Washington, D. C	....	.29	.0012	....
D. F. Fisher, Philadelphia, Pa	51.36	.23	.005	.49
	52.43	....	....	.53
H. C. Fuller, Washington, D. C	55.4	.22	.002	.51
	....	.22	....	.53
C. H. Kimberly, Philadelphia, Pa	44.4	.21	.004	.47
	48.46	....	....	.49
	50.52	....	....	....
	50.09	....	....	....
C. B. Morrison, New Haven, Conn	53.12	.23	[1] Positive.	.54
	53.05	.24	....	.54
E. K. Nelson, Washington, D. C	51.01	.22	.0013	.53
	....	.22	....	.53
A. E. Paul, Chicago, Ill	....	.21	.002(?)	.56
A. Seidell, Washington, D. C	52.7	.23	.0011	.53
	52.4	....	....	....
	49.0	....	....	....
	48.9	....	....	....

[1] 4. 4 mg found.

[As will be seen, with the exception of Emery's figures, the results obtained in the determination of caffein agree very well, and those for phosphoric acid are also fairly concordant. The results for cocain show that it is doubtful if the small quantity present can be determined with absolute accuracy, though in proportion to the whole mixture the results obtained are fair. The method for determining total solids evidently needs revision, and the consensus of opinion was that 10 grams are sufficient for a sample instead of 25 cc as recommended in the method, and the residue should not be heated above 100° C. It is suggested that this work be continued during the ensuing year.]

ESTIMATING SMALL QUANTITIES OF MORPHIN IN MIXTURES.

By E. O. Eaton.

At the last meeting the writer reported (Bul. 137, p. 188) a method for estimating small quantities of morphin in mixtures. During the past year this method was tried on the following samples:

Powdered opium: 10.77 per cent morphin U. S. P. assay.

Paregoric: U. S. P. preparations made up with 9 grams (10.77 per cent U. S. P. assay) opium to 2 liters; 100 cc=48.46 mg morphin.

Special sirup.

Sirup (U. S. P.) (cc)	1,000
Glycerin (cc)	100
Alcohol (cc)	100
Morphin hydrochlorid (grams)	1.08
Sodium bicarbonate (grams)	0.10
Oils of anise and peppermint, equal parts (cc)	0.5
Water sufficient to make (cc)	2,000

100 cc=43.58 mg morphin.

RESULTS OBTAINED.

Powdered opium.

	Per cent.
E. O. Eaton	11
H. A. Seil	12.3
A. E. Stevenson	9.96 9.72 10.02
H. E. Buchbinder	10.80

Paregoric.

	Mg per 100 cc.
E. O. Eaton	48.00
H. A. Seil	51.10
A. E. Stevenson	36.00 30.90
H. E. Buchbinder	49.20

Special sirup.

	Mg per 100 cc.
E. O. Eaton	39.7
H. A. Seil	38.5
A. E. Stevenson	28.8 30.9
H. E. Buchbinder	37.8

Three workers obtained fairly satisfactory results in the case of the paregoric and the special sirup. Stevenson's results plainly show difficulty somewhere. The results on opium are unsatisfactory. The reasons for the discrepancies in the latter are not plainly evident.

COMMENTS BY ANALYSTS.

H. A. Seil: On acetylating the residue obtained from the powdered opium and determining the morphin, 11.21 per cent was obtained.

A. E. Stevenson: In the assay of opium it required about seven washings to completely remove the uncombined alkaloids from the lime water solution. In both the paregoric and the opium it was found that about 50 per cent more alcohol-chloroform mixture than directed was required to extract the morphin. In each case the final residue was gummy and quite dark in color; it was difficult to dissolve in the fiftieth-normal acid and gave solutions of such color that the titration was finished with difficulty.

H. E. Buchbinder: I found from numerous experiments that the number of shake outs prescribed both in the first stage when the lime water is shaken out and later when the morphin is extracted is seldom sufficient. Would recommend:

1. That in both stages a minimum of seven extractions be made and that a small part of the last shake out be tested for alkaloids. If a reaction results the extraction is to be continued until a negative test is obtained.
2. If the alcohol-chloroform shake out is anything but a very light yellow it should be washed with small portions of water and the water in turn washed with suitable quantities of alcohol-chloroform, the latter to be added to the main alcohol-chloroform solution.
3. In place of cochineal as indicated use methyl red (three drops) as indicator; the change in color is very sharp and is only slightly affected by coloring matter. Moderate dilution does not affect the end point to the same extent as when cochineal is used.
4. Before adding standard acid dissolve in about 5 cc of warm neutral alcohol.

RECOMMENDATIONS.

It is recommended that the method as modified below be tried during the coming year. On page 189 of Bulletin 137, line 13, of method for opium, insert, "be sure to extract all of the morphin, using more shake outs, with alcohol and choloroform if necessary." Line 15, strike out word "tared" before "beaker" and "150 cc capacity" after beaker.

STANDARD FOR TINCTURE OF GINGER.

By L. F. KEBLER and C. H. KIMBERLY.

The United States Pharmacopœia, eighth revision, gives a method for making tincture of ginger, but does not prescribe a definite standard for the finished product. Since all commercial varieties are derived from the same species and the variation in quality is due entirely to different conditions of climate, soil, cultivation, etc., the Pharmacopœia places no restrictions whatsoever upon ginger when it says that ginger is the rhizome of *Zingiber officinalis* (Roscoe), and it is construed to mean that any available ginger may be employed in preparing the tincture. It is well known that the various gingers differ materially in aroma and pungency. These points are, however, not considered in this study.

PRELIMINARY EXAMINATIONS.

The names "tincture of ginger" and "extract of ginger" are frequently used interchangeably. An examination of a number of these products available on the market gave the following results:

Analytical data on ginger extracts and tinctures.

No.	Alcohol.	Residue.	Ash.	Capsicum.	Artificially colored.
	Per cent.	*Per cent.*	*Per cent.*		
1	5.53	1.27	0.28	Present...	Yes.
2	56.76	.70	.15	...do.......	No.
3	87.00	2.86	.095	...do.......	Yes.
4	90.00	1.22	1.021	None......	No.
5	10.00	5.40	.048	Trace.....	Yes.
6	24.00	3.30	.009	None......	Yes.
7	70.80	3.84	.009	None	No.

The alcohol was determined by three methods, as follows:

(1) Dilute to three times the original volume and distil two volumes; determine specific gravity at 25° C. (2) Dilute to three volumes with saturated salt solution, shake out with petrolic ether, boiling point not above 60° C., discard the petrolic ether and distil two volumes; determine specific gravity at 25° C. (3) Add 10 cc of water and distil carefully to original volume. Method 2 is by far the best.

In some instances it was clearly indicated that the product was intended for medicinal purposes, but it was rarely stated that the article deviated from one prepared by the Pharmacopœial process. The results also show that a number of the preparations contain capsicum and are artificially colored. The amount of alcohol contained in Nos. 3 and 4 indicated that these samples were made with alcohol of proper strength and in accordance with the Pharmacopœial process, but the large amount of nonvolatile matter in No. 3 and the high per cent of ash in No. 4, together with the fact that No. 3 was artificially colored and contained capsicum raised the question as to a specific standard for judging the quality of tincture of ginger. Apparently other workers have been in doubt as to constants for extract and tincture of ginger. Their work deals, however, more with ginger extract.[1]

A number of samples of the tincture, representing the most prominent manufacturers, were then purchased and examined. There were also prepared in the laboratory according to the Pharmacopœial process two samples of tincture, one made with African ginger (No. 8) and the other with Jamaica ginger (No. 7). An examination of these samples gave the following results:

Examination of commercial and laboratory tinctures of ginger.

No.	Specific gravity at 25° C.	Alcohol claimed.	Alcohol found.	Non-volatile matter.	Ash.
		Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
1	0.8220	94	90	1.26	0.0075
2	.8201	90	90	1.12	.0066
3	.8144	95	91	1.25	.0068
4	.8184	88	90	1.60	.0074
5	.8271	91.2	87	1.27	.0049
6	.8210	89	89.5	1.59	.0145
[1] 7	.8140	U. S. P.	92	1.28	.0069
[1] 8	.8142	U. S. P.	90.5	1.36	.0062

[1] Made in laboratory.

Collection of Powdered Ginger Samples and Determination of Degree of Fineness.

The results obtained were still considered too meager to warrant the deduction of any definite standards for tincture of ginger. Arrangements were then made to procure samples of powdered ginger prepared from the various commercial varieties. As far as it was possible a sample of the whole rhizome from which the powdered gingers were prepared was also obtained. The uses to which ginger is put seem to demand different degrees of fineness; the condimental ginger being ground comparatively fine, while that used for Pharmacopœial purposes is usually coarsely ground, in fact, much coarser than is prescribed by the United States Pharmacopœia, eighth edition, which calls for a No. 50 powder. The degrees of fineness to which the samples obtained were ground were claimed to give the greatest satisfaction to drug firms who used them. Samples of the following varieties of ginger rhizome together with the powdered ginger or the ground ginger prepared therefrom were procured:

1. *African ginger.*—Unpeeled; dark brown color; fracture discolored in spots; odor good; taste sharp and pungent; rhizome small but of fair quality; the powder darker, finer, and more fibrous than sample No. 5, but lighter and coarser than No. 2.
2. *Jamaica ginger.*—Peeled, washed, and well dried; light yellow in color; brittle mealy fracture; odor slightly starchy; taste pungent. Powder derived from the above is quite fibrous, light colored, aromatic, and pungent.
3. *Cochin ginger.*—Unpeeled, dark, earthy appearing, with some soil attached, some rhizomes brittle, others tough; odor and taste correct; fiber showing in fracture. Powdered sample from above presents a very good appearance with but little fiber showing; color straw amber; quite finely ground; odor and taste good.
4. *Jamaica ginger.*—Peeled rhizomes larger and darker colored than Jamaica No. 2; apparently but little washed; very brittle in fracture; quite mealy;

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[1] Street and Morrison, U. S. Dept. Agr., Bureau of Chemistry Bul. 137, p. 76; Lythgoe and Nurenberg, J. Ind. Eng. Chem., 1911, 3: 910.

odor cereal-like or starchy; taste aromatic and pungent; powder light in color, finely ground; color, odor, and taste good.

5. *African ginger.*—Unpeeled; very dark; roughly wrinkled, and rhizomes bony and coarse; fracture brittle, mealy; odor strong; usual pungent taste. Powder medium fine; color brown, not different from usual.

6. *Jamaica ginger.*—Peeled, washed; very light yellow; quite large rhizomes; odor like cereal; taste normal; finely ground; taste, color, and odor normal.

7. *Calcutta ginger.*—Very coarse; dark brown; almost black in places where cortex had been removed. Internally light brownish yellow; brittle; mealy, and showing bundles of fiber; odor and taste natural but weak. Ground sample quite dark and coarse, odor and taste normal.

8. *Calcutta ginger.*—Coarse; unpeeled; dirty, and more or less wormy; dark grayish brown. Sample powdered produced a dark powder quite finely ground, showing few fibers.

9 and 10. *Cochin ginger.*—Large; partially peeled rhizomes; very light yellow; some pieces darker; fracture brittle; odor and taste strong but natural. (9) Ground; represents practically No. 20 powder, being granulated about the size of ground coffee. This sample was also screened during grinding in order to remove dust and dirt; (10) finely ground for condiment purposes; light color; some fiber showing, odor and taste normal.

11 and 12. *African ginger.*—Dark brown, but lighter than African No. 1; unpeeled, except in spots; fracture brittle; odor and taste normal. (11) Ground to No. 20 fineness with its characteristics; (12) finely ground; brownish; dry; odor and taste acrid pungent.

13 and 14. *Japanese ginger.*—Decorticated; washed and limed, but not heavily; rhizome small; odor good, aromatic. (13) Coarsely ground, No. 20. (14) Finely ground; trifle darker than Jamaica, less fiber; odor and taste normal.

15 and 16. *Jamaica ginger.*—Peeled, washed, very light in color; small rhizome; odor and taste normal but strong; some dark rhizomes; fracture brittle; fibrous; odor and taste good. (15) Ground coarse, No. 20; (16) finely ground, showing less fiber; color light yellow; odor and taste normal.

17 and 18. *Calcutta ginger.*—Dark color; cortex coarse, thick; some soil attached. (17) Ground about No. 20, and screened during grinding; (18) ground very fine for condimental purposes, not screened. All samples ground to No. 20 were screened in grinding so that some of the earthy matter was removed. The more finely ground samples were not screened.

In order to determine the degree of fineness, samples were sifted through wire mesh sieves of standard size, using 150 grams in each case with the following results:

Determination of degree of fineness, using 150-gram samples.

No.	50 mesh.	40 mesh.	30 mesh.	20 mesh.	10 mesh.	Residue.	Remarks.
	Grams.	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	
1	52	52	35	11			Fibrous.
2	30	31	27	31		30	Fibrous.
3	23	36	35	27		29	Fibrous.
4	52	52	35	11			
5	82	32	26	10			
6	71	21	25	24		9	Few fibers.
7	45	22	30	53		Trace.	Trace fiber.
8	112	38					Very finely ground.
9[1]	12		45	30	63		
10[2]	30	120				Trace.	

[1] Nos. 11, 13, 15, and 17 were ground and sieved in same manner as No. 9.

[2] Nos. 12, 14, 16, and 18 were ground in same manner as No. 10.

These results clearly show that there is little powdered ginger supplied which complies with the degree of fineness prescribed by the Pharmacopœia (No. 50). What bearing this may have upon the finished product will be considered in connection with the results obtained in the examination of the tinctures made from the various samples.

EXAMINATION OF POWDERED SAMPLES.

In order to determine whether or not the samples complied in quality with the standards set for ginger by the committee on food standards (U. S. Dept. Agr., Office of Secretary, Cir. 19), it was decided to examine each sample by the official methods published in Bulletin 107, Revised. Wherever deviations from these methods were made, the fact is clearly indicated. A convenient quantity in the condition in which it was obtained was burned free from carbon, using as low a temperature as possible. The ash was boiled with 25 cc of 10 per cent hydrochloric acid, specific gravity 1.050, for five minutes, the insoluble material was collected in a Gooch crucible, washed with hot water, ignited, and weighed. The iron and aluminum were precipitated by ammonium hydroxid, and the quantity merely noted by comparison. The filtrate left after removing the above was heated to 50° C., rendered acid, if not acid, with acetic acid, and ammonium oxalate added. The calcium oxalate was filtered, washed, ignited, and then blasted to convert it into calcium oxid, and weighed.

The cold water extractive was determined by placing 4 grams in a 200 cc flask; water added to the mark, shaken at intervals of half an hour during eight hours' time, and allowed to stand 16 hours without shaking; 50 cc of the resulting fluid were transferred to a flat-bottomed dish and dried to constant weight at 100° C. The vacuum was not available, but a definite temperature on the water bath was maintained. The ether extract was determined by using anhydrous ether in a continuous extraction apparatus; extraction was continued 20 hours. The results obtained are given in the following table:

Analysis of the 18 powdered samples.

No.	Ash.	Insoluble.	Lime.	Water extract.	Starch.	Crude fiber.	Ether extract.	Aluminum + iron.	Remarks.
1	5.84	0.91	0.22	11.84	43.29	7.60	8.36	Trace	African.
2	5.05	.43	.28	15.42	47.43	5.95	5.83	do	Jamaica.
3	5.66	.70	.24	14.34	42.07	7.56	8.31		Cochin.
4	3.64	.48	.23	12.61	60.30	4.89	4.32	Trace	Jamaica.
5	5.14	.44	.16	13.37	43.83	6.89	7.30		African.
6	4.00	.40	.14	13.75	57.80	3.37	4.20	Trace	Jamaica.
7	7.34	3.02	.15	11.01	39.15	5.14	4.90		Calcutta.
8	9.79	3.65	.25	14.31	32.12	7.02	8.11		Do.
9	5.83	.83	.34	11.98	51.23	6.91	7.52		Cochin.
10	6.39	.96	.32	15.97	54.45	4.50	6.57		Do.
11	5.28	1.43	.10	10.73	45.18	3.90	6.37	Trace	African.
12	5.46	1.28	.11	12.50	51.39	7.16	7.49		Do.
13	5.31	.58	.88	11.42	51.39	6.40	4.57	Trace	Japanese.
14	5.95	.54	1.56	13.57	55.08	3.80	6.26		Do.
15	3.41	.32	.14	12.22	55.44	3.72	3.25	Trace	Jamaica.
16	3.92	.28	.14	14.20	57.78	2.72	4.62		Calcutta.
17	5.85	1.04	.06	10.57	34.12	7.90	8.89		Do.
18	7.18	2.06	.15	12.87	32.02	6.45	6.79		Do.
	6.0	3.0	1.00		42.00	8.00			Standard.
	10.0		4.00		42.00	8.00			Limed.

The Calcutta ginger and one Cochin ginger run high in ash. The others are all within the limits of the standard. The Calcutta ginger has an excess of insoluble ash. The lime content of one sample of Japanese ginger was high but considerably below the limit allowed for limed ginger. All samples were within the limits prescribed for crude fiber and starch, excepting the Calcuttas.

TINCTURES PREPARED FROM POWDERED SAMPLES.

Tinctures were next prepared from these samples in accordance with the Pharmacopœial instructions, and their characteristics observed after standing six months. The reason for making the observations at the end of six months is that during this time the physical characteristics of many galenical preparations change materially. Tincture of ginger, however, is not apt to change very much. The points taken into consideration were sediment, color, specific gravity, residue, nonvolatile matter, and ash. The constants were determined in the usual manner. Color, however, was made a comparative determination by the use of a colorimeter, one of the samples of medium color being taken as a standard, and a length of column equal to 20 mm used. The numbers opposite the color in the table, therefore, represent the length of column of that sample which gave approximately the same color as the sample used as a standard.

Tinctures from powdered samples examined after standing for 6 months.

No.	Non-volatile.	Ash.	Sediment.	Color.	Specific gravity.
1..	Broken....				
2..	1.40	0.0055	None......	Red-38	0.8128
3..	1.89	.0060	...do.......	Red-27	.8147
4..					
5..	1.56	.0050	None......	Dark-8	.8128
6..	1.13	.0012	...do.......	Light-62	.8133
7..	1.04	.0030	...do.......	Dark-18	.8116
8..					
9..	1.44	.0030	None......	Medium-20	.8157
10..	1.19	.0080	...do.......	Darker-14	.8130
11..	1.29	.0050	...do.......	Deep red-16	.8133
12..	1.82	.0050	...do.......	Deep red-16	.8147
13..	.99	.0100	...do.......	Amber red-46	.8131
14..	1.75	.0100	Slight.....	Deep red-18	.8145
15..	.89	.0040	...do.......	Very light-59	.8125
16..	1.40	.0060	None......	Red brown-34	.8132
17..	.96	.0012	...do.......	Red brown-29	.8134
18..	1.31	.0012	...do.......	Red brown-17	.8134

These results corroborate the observations already made, namely, that the Calcutta and Japanese gingers are not suited for the manufacture of tincture of ginger. The degrees of fineness within the limits used in these investigations do not materially influence the amount of nonvolatile matter abstracted. These results clearly show that a good tincture of ginger should comply with the following requirements: Specific gravity at 25° C. not above 0.8270; alcohol, by volume, not less than 90 per cent; nonvolatile matter from 1.25 to 1.75 per cent. The figures clearly show that tincture of ginger made from Calcutta and Japanese gingers is of an inferior character. The tinctures made from Nos. 6 and 10 also contain a little less nonvolatile matter than the mean, but this is not sufficient to warrant more than a casual comment.

NOTE ON ASSAY OF NITROGLYCERIN TABLETS.

By A. G. Murray.

The methods which have been proposed for the determination of nitroglycerin are (1) the nitrometer method; (2) direct weighing of the ether extract; (3) saponification, and (4) the colorimetric method proposed by W. A. Scoville,[1] which is an adaptation of the well known method for nitrates in water analysis.

The nitrometer method is not well adapted to the estimation of nitroglycerin in medicinal tablets, on account of the small quantity present and the difficulty of introducing it quantitatively, and free from objectionable extraneous matter, into the nitrometer.

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[1] Amer. J. Pharm., 1911, 83: 359.

Weighing the ether extract, where proper precautions to prevent loss are observed, yields entirely satisfactory results, provided, of course, the sample contains no other ether-soluble material.

Discussing the saponification method, Scoville calls attention to the discordant results recorded in the literature by Rice[1], Hay[2], and Beryl and Delpy[3], and adds: "One has only to try the process, varying the conditions of heating, the time of standing, and the temperature, to become convinced that the results are of no value. Indeed, one is surprised to note how much variation in results is induced by slight variations in the process."

The differences in the results recorded by the authors mentioned are easily explained. Rice, who claimed that 3 molecules of potassium hydroxid are required to decompose 1 molecule of nitroglycerin, examined two commercial 10 per cent solutions, obtaining by his method 14.46, and 13.76 per cent respectively, and for six commercial 1 per cent samples he obtained results ranging from 1.18 to 1.81 per cent. He then diluted one of the 10 per cent samples so that, according to his analysis, it would contain 1 per cent. Duplicate analyses of this product by the same method of analysis gave 0.992 and 1.013 per cent, "which figures" the author concluded "prove the reliability of the method." There is no indication that the method was tried on a sample of known nitroglycerin content. Further consideration of this method is, therefore, unnecessary. The apparently high results obtained, if of any value, tend to prove that considerably more than 3 molecules of caustic potash are required to saponify 1 molecule of nitroglycerin.

Beryl and Delpy, who state that each molecule of nitroglycerin requires 6 molecules of potassium hydroxid, record only one experiment which they describe as follows: "Each 5 cc glycerin trinitrate was diluted with absolute ethyl alcohol to 100 cc, cooled to 4° and * * * slowly added to 100 cc alcoholic potash (10.5 grams potassium hydroxid in 100 cc 90 per cent alcohol). For each molecule of glycerin trinitrate there are about six molecules potassium hydroxid."

Considering the specific gravity of nitroglycerin to be 1.6 (Perkin, J. Chem. Soc., 1889, 55: 685), and assuming that the potassium hydroxid used was 100 per cent, the quantities used would represent 1 molecule nitroglycerin to 5.3 molecules potassium hydroxid, while if it is assumed that the alkali was 85 per cent, which is about the content of the grade usually found on the market for analytical purposes, the ratio of nitroglycerin to hydroxid is about 1 to 4.5. In view of what follows, it will be seen that there was not an excess of alkali, as stated by Berl and Delpy, and this is a possible explanation of the presence of glycerin dinitrate and unchanged trinitrate found in the reaction products.

The experiments of Hay were for the purpose of determining the nitrite produced by the saponification of nitroglycerin. The results of many experiments carried out under varying conditions show conclusively that the proportion of nitrite produced is sufficiently constant and independent of minor variations in the conditions to warrant the use of the method for quantitative estimations. Experiments are recorded by Hay in which the conditions varied from an excess of alkali to an excess of nitroglycerin, and from boiling the alcoholic solution on the steam bath for 30 minutes to saponifying in the cold for 2 minutes. The yield of nitrite varied within narrow limits. Hay showed that approximately 5 molecules of potassium hydroxid are necessary to saponify 1 molecule of nitroglycerin.

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[1] Amer. Druggist, 1895, 27: 6.

[2] Trans. Royal Soc. Edinburgh, 1885, 32: 67.

[3] Ber. d. chem. Ges., 1910, 43: 1421.

The method of estimation proposed by Hay depends on the determination of the nitrite produced by saponification, not on a titration with standard alcoholic potash and standard acid. Since small amounts of nitrous acid can be readily and accurately estimated, Hay's method is well adapted to the estimation of small quantities of nitroglycerin.

Scoville proposes to estimate nitroglycerin in tablets by evaporating the alcoholic extract, treating the residue with phenoldisulphonic acid, and determining the nitrate colorimetrically. The method is simple and gives excellent results. The substitution of ether for alcohol as a solvent improves the method. Ether can be rapidly evaporated in a vacuum. The temperature falls below zero, thus preventing any volatilization of the nitroglycerin with the ether. Ether does not dissolve any of the sugar usually present in the tablets, which is a decided advantage over alcohol, as the action of the sulphonic acid reagent on sugar is apt to produce a coloration which interferes with the accuracy of the test. Ethereal solutions do not require filtering, since the insoluble material settles very rapidly and the supernatant clear solution can be drawn off with a pipette. With alcohol the insoluble matter settles much more slowly. Sodium nitrate, which might be present as a decomposition product of nitroglycerin or as an accidental impurity, is insoluble in ether, but is soluble in alcohol and would be estimated as nitroglycerin.

There are, then, two methods suitable for the estimation of minute quantities of nitroglycerin. The weight of the ether extract and the determination of total nitrogen by the modified Gunning method frequently give additional useful information. The following outline is suggested as a convenient procedure in the analysis of nitroglycerin tablets:

Crush 25 tablets under 10 cc ether. A 25 cc cylindrical graduate makes a convenient container for the purpose. Decant the ether into a 50 cc graduated flask and wash the residue repeatedly with 5 cc portions of ether, finally making the solution up to the mark. It is immaterial if a little of the insoluble matter gets into the flask. Mix the solution thoroughly, allow any insoluble matter to settle, and carefully remove 20 cc of the clear solution with a pipette and place in a small tared dish or beaker. Place the beaker in a vacuum desiccator. With a good vacuum the evaporation will require only an hour or two. Weigh the residue. Treat with 2 cc of phenoldisulphonic acid reagent[1] and after 5 to 10 minutes dilute, wash into a 100 cc graduated flask, dilute to the mark, mix well, withdraw 10 cc representing 1 tablet, dilute nearly to 100 cc, neutralize with a few drops potassium hydroxid solution, and complete the volume to 100 cc. Compare the color with that produced by a standard nitrate solution similarly treated. Nitroglycerin is calculated by multiplying the nitrogen by 5.4.

To 5 cc of the ethereal solution add 5 cc of 0.5 per cent alcoholic potassium hydroxid, place on steam bath and allow most of the alcohol to boil off, add water and leave on the bath until the odor of alcohol is no longer perceptible. Dilute to 250 cc. Mix and place an aliquot representing 0.02 to 0.04 mg of nitroglycerin in a 100 cc graduated flask, add about 50 cc of water, 1 drop of concentrated hydrochloric acid, 2 cc of 1 per cent sulphanilic acid solution and 2 cc of 0.5 per cent naphthylamin hydrochlorid solution. Dilute to 100 cc and after 30 minutes compare the color with standard nitrite solutions similarly treated. Nitroglycerin is calculated by multiplying nitrite nitrogen found by 8.

The association adjourned.

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[1] Details of the preparation of this reagent as given by Chamot, Pratt, and Redfield, J. Amer. Chem. Soc., 1911, 33: 382, may be followed.

OFFICERS AND REFEREES OF THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS, 1911-12.

President.

H. J. PATTERSON, College Park, Md.

Vice President.

G. S. FRAPS, College Station, Tex.

Secretary.

H. W. WILEY, Washington, D. C.

Additional members of the executive committee.

R. E. DOOLITTLE, Washington, D. C. A. J. PATTEN, East Lansing, Mich.

Referees.

Phosphoric acid: H. D. Haskins, Amherst, Mass.
Nitrogen:
  Determination of nitrogen: C. L. Hare, Auburn, Ala.
  Separation of nitrogenous bodies (meat proteids): A. D. Emmett, Urbana, Ill.
Potash: H. B. McDonnell, College Park, Md.
Soils: G. S. Fraps, College Station, Tex.
Dairy products: E. M. Bailey, New Haven, Conn.
Foods and feeding stuffs: W. J. Jones, jr., La Fayette, Ind.
Food adulteration: A. S. Mitchell, St. Paul, Minn.
Sugar: W. E. Cross, Audubon Park, New Orleans, La.
Tannin: J. S. Rogers, Washington, D. C.
Insecticides: S. D. Averitt, Lexington, Ky.
Inorganic plant constituents: W. H. McIntire, Knoxville, Tenn.
Medicinal plants and drugs: L. F. Kebler, Washington, D. C.
Water: W. W. Skinner, Washington, D. C.

Associate referees.

Phosphoric acid: A. J. Patten, East Lansing, Mich.
Nitrogen:
  Determination of nitrogen: B. L. Hartwell, Kingston, R. I.
  Separation of nitrogenous bodies:
    Milk and cheese: A. W. Bosworth, Geneva, N. Y.
    Vegetable proteids: T. B. Osborne, New Haven, Conn.
Potash:
  Determination: W. E. Tottingham, Madison, Wis.
  Availability: E. E. Vanatta, Columbia, Mo.
Soils: J. H. Pettit, Urbana, Ill.
Dairy products: L. I. Nurenberg, Boston, Mass.
Foods and feeding stuffs: C. S. Cathcart, New Brunswick, N. J.
Food adulteration:
  Colors: W. E. Mathewson, New York, N. Y.
  Saccharine products: J. R. Chittick, Des Moines, Iowa.
  Fruit products: H. C. Gore, Washington, D. C.
  Wine: E. J. Lea, Berkeley, Cal.
  Beer: Julius Hortvet, St. Paul, Minn.

Food adulteration—Continued.
Distilled liquors: J. O. La Bach, Lexington, Ky.
Vinegar: W. A. Bender, New York, N. Y.
Flavoring extracts: R. S. Hiltner, Denver, Colo.
Spices: R. W. Hilts, Philadelphia, Pa.
Baking powder: Edmund Clark, Boston, Mass.
Meat and fish: W. B. Smith, Kansas City, Mo.
Fats and oils: H. S. Bailey, Washington, D. C.
Dairy products: A. E. Paul, Chicago, Ill.
Cereal products: H. L. White, Agricultural College, N. Dak.
Vegetables: J. P. Street, New Haven, Conn.
Condiments other than spices: W. J. McGee, New Orleans, La.
Cocoa and cocoa products: W. L. Dubois, Buffalo, N. Y.
Tea and coffee: M. E. Jaffa, Berkeley, Cal.
Preservatives: H. E. Barnard, Indianapolis, Ind.
Water in foods: H. C. Lythgoe, Boston, Mass.
Organic and inorganic phosphorus in foods: E. B. Forbes, Wooster, Ohio.
Heavy metals in foods: H. M. Loomis, Seattle, Wash.
Sugar: M. N. Straughn, Washington, D. C.
Insecticides: R. C. Roark, Washington, D. C.
Medicinal plants and drugs:
Synthetic products: W. O. Emery, Washington, D. C.
Medicated soft drinks: G. W. Hoover, Washington, D. C.
Medicinal plants: A. E. Ruddiman, Nashville, Tenn., and H. H. Rusby, New York, N. Y.
Water: G. E. Colby, Berkeley, Cal.
Tannin: C. B. Bacon, Washington, D. C.
Inorganic plant constituents: B. E. Curry, Durham, N. H.

SPECIAL COMMITTEES.

Amendments to the constitution:

L. L. Van Slyke, Geneva, N. Y., chairman.
B. B. Ross, Auburn, Ala.
William Frear, State College, Pa.

Appropriation:

John Phillips Street, New Haven, Conn., chairman.
R. J. Davidson, Blacksburg, Va.
H. E. Barnard, Indianapolis, Ind.

Availability of phosphoric acid in basic slag:

C. B. Williams, West Raleigh, N. C., chairman.
C. G. Hopkins, Urbana, Ill.
H. D. Haskins, Amherst, Mass.
John S. Burd, Berkeley, Cal.
B. L. Hartwell, Kingston, R. I.

Food standards:

William Frear, State College, Pa., chairman.
H. W. Wiley, Washington, D. C.
H. A. Weber, Columbus, Ohio.
M. A. Scovell, Lexington, Ky.
E. H. Jenkins, New Haven, Conn.

Presentation of the question of unification of terms to the International Congress of Applied Chemistry:

R. J. Davidson, Blacksburg, Va., chairman.
C. G. Hopkins, Urbana, Ill.
W. D. Bigelow, Washington, D. C.
G. S. Fraps, College Station, Tex.
B. W. Kilgore, Raleigh, N. C.
H. J. Wheeler, Kingston, R. I.
J. T. Willard, Manhattan, Kans.

Recommendation of referees and revision of methods.

[Figures in parentheses refer to year in which appointment expires.]

A. L. WINTON, chairman.

SUBCOMMITTEE A: W. W. Skinner (1916), B. B. Ross (1914), *J. P. Street (1912), chairman, Agricultural Experiment Station, New Haven, Conn.*

SUBCOMMITTEE B: H. E. Barnard (1916), *E. M. Chace (1914), chairman, Bureau of Chemistry, U. S. Department of Agriculture, Washington, D. C.*, J. M. Bartlett (1912).

SUBCOMMITTEE C: B. F. Trowbridge (1916); C. D. HOWARD (1914), *A. L. Winton (1912), chairman, U. S. Food and Drug Inspection Laboratory, Chicago, Ill.*

Testing of chemical reagents:

L. F. Kebler, Washington, D. C., chairman.
A. L. Winton, Chicago, Ill.
B. W. Kilgore, Raleigh, N. C.

Unification of methods of analysis of fats and oils.

L. M. Tolman, Washington, D. C., chairman.
P. H. Walker, Washington, D. C.
A. Lowenstein, Chicago, Ill.

CONSTITUTION OF THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS.

(1) This association shall be known as the Association of Official Agricultural Chemists of North America. The objects of the association shall be (1) to secure uniformity and accuracy in the methods, results, and modes of statement of analysis of fertilizers, soils, cattle food, dairy products, human foods, medicinal plants, drugs, and other materials connected with agricultural industry; (2) to afford opportunity for the discussion of matters of interest to agricultural chemists.

(2) Analytical chemists connected with the United States Department of Agriculture, or with any State, provincial, or national agricultural experiment station or agricultural college, or with any State, provincial, or national institution or body in North America charged with official control of the materials named in section 1, shall alone be eligible to membership; and one such representative for each of these institutions or boards, when properly accredited, shall be entitled to enter motions or vote in the association. Only such chemists as are connected with institutions exercising official fertilizer control shall vote on questions involving methods of analyzing fertilizers or involving definitions, nomenclature, laws, or regulations relating to fertilizers. Only such

chemists as are connected with institutions exercising official cattle-food control shall vote on questions involving methods of analyzing cattle foods or involving nomenclature, definitions, laws, or regulations relating to cattle foods. Only such chemists as are connected with institutions exercising official food or drug control shall vote on questions involving methods of analyzing food or drugs or involving nomenclature, definitions, laws, or regulations relating to food or drugs. All persons eligible to membership shall become members ex officio and shall be allowed the privileges of membership at any meeting of the association after presenting proper credentials. All members of the association who lose their right to such membership by retiring from positions indicated as requisite for membership shall be entitled to become honorary members and to have all privileges of membership save the right to hold office and vote. All analytical chemists and others interested in the objects of the association may attend its meetings and take part in its discussions, but shall not be entitled to enter motions or vote.

(3) The officers of the association shall consist of a president, a vice president, and a secretary, who shall also act as treasurer, and these officers, together with two other members to be elected by the association, shall constitute the executive committee. When any officer ceases to be a member by reason of withdrawing from a department or board whose members are eligible to membership, his office shall be considered vacant, and a successor may be appointed by the executive committee, to continue in office till the annual meeting next following.

(4) There shall be appointed by the executive committee, at the regular annual meeting, from among the members of the association, a referee and such associate referees for each of the subjects to be considered by the association as that committee may deem appropriate. [Construed by resolution passed in 1911 to mean the outgoing executive committee; standing rule adopted that the committee consult with each referee in the appointment of associates.]

It shall be the duty of these referees to prepare and distribute samples and standard reagents to members of the association and others desiring the same, to furnish blanks for tabulating analyses, and to present at the annual meeting the results of work done, discussion thereof, and recommendations of methods to be followed.

(5) The special duties of the officers of the association shall be further defined, when necessary, by the executive committee.

(6) The annual meeting of this association shall be held at such place as shall be decided by the association, and at such time as shall be decided by the executive committee, and announced at least three months before the time of meeting.

(7) No changes shall be made in the methods of analysis used in official inspection, except by unanimous consent, until an opportunity shall have been given all official chemists having charge of the particular inspection affected to test the proposed changes.

(8) Special meetings shall be called by the executive committee when in its judgment it shall be necessary, or on the written request of five members; and at any meeting, regular or special, seven enrolled members entitled to vote shall constitute a quorum for the transaction of business.

(9) The executive committee will confer with the official boards represented with reference to the payment of expenses connected with the meetings and publication of the proceedings of the association.

(10) All proposed alterations or amendments to this constitution shall be referred to a select committee of three at a regular meeting, and after report from such committee may be adopted by the approval of two-thirds of the members present entitled to vote.

BY-LAWS.

(1) Any amendment to these by-laws or addition thereto may be proposed at a meeting of the association and shall be published in the Proceedings. It may then be adopted by a majority vote of the association at the next meeting.

(2) These by-laws or any portion of them may be suspended without previous notice by a unanimous vote of those present at any meeting of the association.

(3) There shall be a committee of nine members which shall be designated as the committee on recommendations of referees. The president shall appoint three members of this committee to serve six years, such appointments to be made every other year as the terms expire. The chairman of the committee shall be appointed by the president and shall divide the nine members into three subcommittees (A, B, and C), and shall assign to each subcommittee the reports and subjects it shall consider.

(4) Each referee shall forward to the chairman of the committee on recommendations at least three weeks before the meeting of the association his recommendations and a sufficient abstract of his report to enable the committee to act intelligently on the recommendations.

As soon as possible after the annual meeting of the association each retiring referee shall transmit a copy of his report and recommendations, together with a statement of the action taken by the association upon the same, to the referee for the next year. [1911.]

(5) A method shall not be adopted as provisional or a provisional method amended until such method or amendment has been reported by the appropriate referee and published in the Proceedings of the association.

(6) A method shall not be adopted as official or an official method amended until such method or amendment has been recommended as official for at least two years by the appropriate referee.

(7) Each college, experiment station, bureau, board, or other institution entitled to representation in the association shall contribute annually $2, and its representatives shall not be qualified to vote or hold office in the association unless such annual dues have been paid, but these shall not be cumulative.

INDEX.

Page.
Acetanilid, determination in headache mixtures 241
Acetphenetidin, determination in headache mixtures 240
Acid, acetic, phosphoric, etc. *See* Acetic acid; Phosphoric acid, etc.
Acids, determination in mineral waters, recommendations 80–81, 82, 83
Adulteration, food, report by A. S. Mitchell, referee 88–89
Affiliated Societies of Agricultural Science, organization, constitution, etc... 170–171
Agricultural organizations, cooperation, report of committee 170–171
  research journal, report of committee, committee discharged, etc. 195
  Science, Affiliated Societies, organization, constitution, etc..... 170–191
Agriculture, Secretary, address to convention, mention 197
Albrech, M. C., comments on ether extract from cocoa products 160
Albumen, determination in flour 104
Alcohol soluble proteids, determination in flour 104
  solutions, essential oil determination, note by R. S. Hiltner 195–196
  tables, standardization, report by L. M. Tolman, as chairman of committee 169
Aluminum, determination in mineral waters 78
Ammonia determination, recommendations 86
  free, and albuminoid, determination in mineral waters 82
  in cheese, determination 186–187
Ammonium citrate, neutral solution, methods of preparation 25–27
Antipyrin, determination in headache mixtures 240
Applied Chemistry, Eighth International Congress, invitation to association to meeting 72
Arsenate, lead, analysis 68–70
Ash determination in flour, methods 102, 107
  ginger tinctures 247–248
  powdered ginger 247
  solution, oxid separation, methods, analysis, etc 60–66
Ashes, wood, potash content, determination 184–185
Associate referees, for 1911–12, directory 251–252
Association, members, names of attendants at meeting 5–9
  of Official Agricultural Chemists, constitution and by-laws 253–255
    officers, referees, and committees for 1911–12 251–253
Attendance, members and visitors, list 5–9
Auditing committee, report 212–213
Aumer, J. P., comment on ferric and aluminic oxids 64
Averitt, S. D., report as associate referee on lead arsenate 69

Bailey, H. C., baking test for flour 105–106, 111
  H. S., comments on ether extract from cocoa products 160
    report as associate referee on fats and oils 96–100
  L. H., comment on ferric oxid 64

Page.
Baker, E. L., report as referee on potash ... 28–42
    resolution on nitrogen determination ... 28
Baking tests, wheat flour ... 105–106, 111–113
Barnard, H. E., report as associate referee on preservatives ... 167
Bartlett, J. M., report as chairman of auditing committee ... 212
Beatty, Cornelius, comments on cobalti-nitrite method ... 33–34
Bender, W. A., report as associate referee on vinegar ... 125–127
Benzaldehyde, determination in liqueurs, note by F. G. Smith ... 192–195
Berry, E. H., and A. L. Winton, article on "Chemical composition of authentic vanilla extracts, analytical methods, and limits of constants". 145–158
    comments on analysis of flavoring extracts ... 133, 142, 143
Bicarbonic acid, determination in mineral waters ... 82
Bidwell, G. L., comments on crude fiber determination ... 201
        fat determination ... 199
Blair, A. W., report as associate referee on fruit and fruit products ... 218–220
Blockey, J. R., comments on leather analysis ... 227–229
Bloomberg, E., comments on flavoring extracts ... 134, 138, 140, 143
Bosworth, A. W., report as associate referee on separation of nitrogenous bodies in milk and cheese ... 185–187
Bowser, Leon T., article on features of cobalti-nitrite method for potassium ... 45–50
    comment on ferric oxid ... 64
    comments on gravimetric method ... 34
        phosphoric acid ... 17
    paper on "Determination of carbon dioxid in soils" ... 56–59
Boyle, M., comments on determination of crude fiber in prepared mustard ... 94
Brinton, C. S., comments on flavoring extracts ... 134, 138
Bryan-Given-Straughn method for the determination of soluble carbohydrates ... 189–190
Buchbinder, H. E., comments on morphin estimation in mixtures ... 243
Burd, J. S., comments on cobalti-nitrite method ... 33
By-laws, Association of Official Agricultural Chemists ... 255

Caffein, determination in coffee, cooperative work ... 164
        headache mixtures ... 240
        soft drinks ... 241–242
Calcium, determination in mineral waters ... 79
Calvin, J. W., work on nitrogen determination ... 177
Cane sugar, polarization, temperature corrections ... 208
Canned fruits analysis, methods, etc ... 165–169
    goods, collaborative work, instructions for ... 214–215
        tin content, determination ... 214
            comments ... 217
                recommendation ... 217
            limit by Food and Drugs Board ... 214
Capsicum, determination in ginger extract ... 137–139
Carbohydrates, soluble, as dextrose, determination in flour ... 103, 109
        Bryan-Given-Straughn method of determination ... 189–190
Carbon dioxid, determination in soils, paper by Leon T. Bowser ... 56–59
Carbonic acid, determination in mineral waters ... 82
Cereal products, report by H. L. White, associate referee ... 101–113
Cereals, recommendations of committee C ... 189–190
Chace, E. M., report as chairman of committee B ... 210–212
Cheese, recommendations ... 86

Page.
Cheese, separation of nitrogenous bodies, report by A. W. Bosworth, associate referee and O. B. Winter........ 185–187
water extract, analysis........ 187
Chlorin, determination in mineral waters........ 81
Clarifying agents, cane products, polarization........ 211–212
Cobalti-nitrite method for potassium estimation, studies........ 42–50
Cocain, determination in soft drinks........ 241–242
Cocoa products, recommendations of committee C........ 188
report by W. L. Dubois, associate referee........ 159–163
recommendations of committee C........ 188
report by W. L. Dubois, associate referee........ 159–163
Codein, determination in headache mixtures........ 239–240
Coffee, determination of caffein, cooperative work........ 164–165
report by M. E. Jaffa as associate referee........ 163–167
Collison, R. C., report on nitrogen determination by E. B. Forbes........ 177
Colors, report by W. E. Mathewson, associate referee........ 122–124
Committee A, report on recommendations of referees........ 83–86
auditing, report........ 412–413
B, report on recommendations of referees........ 210–212
C, report on recommendations of referees........ 188–192
on agricultural research journal, report........ 197
amendments to constitution and by-laws, report........ 86–87
applied chemistry congress, verbal report by chairman........ 87
appropriations, verbal report by chairman........ 87
chemical reagents, testing, verbal report by chairman........ 87
food standards authorized to pay printing bill........ 87
verbal report by chairman........ 87
nominations, report by acting chairman........ 220
resolutions, report........ 213
Committees, appointment and personnel........ 67–68
1911–12, personnel and directory........ 252–253
Condiments other than spices, recommendations of committee C........ 188
report by W J McGee, associate referee........ 118–122
Conover, Courtney, comments on flavoring extracts........ 134, 138, 140, 142
Constitution, amendments, report of committee........ 86–87
Association of Official Agricultural Chemists........ 253–254
Cordials, note by F. G. Smith........ 192–195
Cottonseed meal, fat determination, cooperative work, comments, etc........ 199–200
Cross, William E., report as referee on sugar and molasses........ 202–207
Crude fiber, determination in flour........ 105
prepared mustard, work........ 91–95
Cummins, J. A., comments on determination of caffein in coffee........ 165

Dairy products, adulteration, report by A. E. Paul, associate referee........ 100–101
recommendations of committee C........ 188
Directory, names of members, officers, referees, and committees 5–9, 67–68, 220, 251, 253
Dodge, C. O., comments on flavoring extracts........ 139, 140, 142
Doolittle and Lourie, tin determination in canned goods........ 214
R. E., suggestions on determination of heavy metals in canned goods. 214
Drinks, soft. *See* Soft drinks.
Drugs, medicinal, recommendations........ 235–236
report by L. F. Kebler, referee........ 234–236
recommendation of referee adopted........ 212

Page.
Drushel, W. A., article on potassium estimation 42–45
Dubois, W. L., report as associate referee on cocoa and cocoa products 159–163
Dunton, Leila, comments on methods of determination of moisture and ash in flour 107, 108

Easton, E. O., report on "Estimating small quantities of morphin in mixtures" 242–244
Edestin, determination in flour 104
Egg-noodle tables, Juckenack's, recalculation 116–117
Emery, W. O., report as associate referee on headache mixtures 236–242
Emmett, A. D., report on nitrogen determination 177–178
Ether extract, determination in cocoa products, work 159–163
  examination of flour, methods 102, 109
  of paprika, drying, work 89–90
Extract, paprika, studies 89–91
  See also under name of material.
Extracts, flavoring, report by R. S. Hiltner, associate referee 127–158

Fat, beef, in lard, collaborative work 96–100
  determination, comparison of methods, report of committee 198–200
  extraction from leather by petroleum ether 223
Fats, analysis, unification of methods, report of committee 169–170
  recommendations of committee C 191–192
  report by H. S. Bailey, associate referee 96–100
Faust, T. A., comments on leather analysis 229–230
Feeding stuffs and foods, recommendations of referees 210
  recommendations 201–202
  report by G. M. MacNider, referee 197–202
Feeds, acidity, reference 197–198
  report by G. M. MacNider, referee 197–202
Fiber, crude, determination in flour 105
  prepared mustard, work 91–95
  method comparison, work, comments, etc ... 200–201
Fish, report of Ralph Hoagland, associate referee 213–217
Flavoring extracts, plan of work, analysts, etc 127–128
  recommendations of committee C 190–191
  report by R. S. Hiltner, associate referee 127–158
Flour, acidity and nitrous nitrogen extracted, effects of time and temperature of digestion on amounts, paper by A. L. Winton and A. W. Hansen. 114–116
  baking test 105–106, 111
  moisture content, methods of determination 101–102, 107
  nitrous nitrogen content, determination 113–114
  wheat, analyzing and testing, work 101–114
Food adulteration, associate refereeship, establishment 233
  report by A. S. Mitchell, referee 88–89
  and Drug Board, Federal, limit on tin in canned goods 214
  determination of tin, by A. W. Hansen and L. C. Johnson 117–118
Foods and feeding stuffs, recommendations of referees adopted 210–211
  water determination, report by Herman C. Lythgoe, associate referee. 167–168
Forbes, E. B., work on nitrogen determination 177
Fraps, G. S., and J. G. Lipman, report on soils 50–56
  report on humus studies 52–53
Frear, William, comments on humus studies 54
  report as chairman of resolutions committee 213
  verbal report as chairman food standards committee 87

Page.

Fruit juices, analysis, method 169
  moisture determination, methods 218–220
  products, methods of analysis, article by A. E. Paul 168–170
    moisture determination, methods 218–220
    report by A. W. Blair, associate referee 218–220
  report by A. W. Blair, associate referee 218–220
Fruits, canned, analysis 165–169
    method of analysis 169
Fuller, F. D., comments on fat determination 199
  H. C., report as associate referee on soft drinks 241–242

Gasoline color value, flour, determination, note 103, 109
Gelatin mixtures, analysis, method 169
Ginger, extract. *See* Ginger, tincture.
  extracts, cooperative work 137–139
  powdered, varieties, analysis, quality determination, etc 245–247
  samples for analysis, description 137
  tincture, analysis, method, etc 244–245
    standard, paper by L. F. Kebler and C. H. Kimberly 244–248
  tinctures, preparation from powdered ginger, analyses 247–248
Gliadin, determination in flour 104
Globulin, determination in flour 104
Glucose determination, leather 222, 224–225, 227, 228, 229–230, 231
Gluten, moist, determination in flour 104–105, 111
Glutenin, determination in flour 104
Grant, E. H., comments on flavoring extracts 134, 138, 143
Griess-Ilosvay method for determining nitrous nitrogen in flour 113–114

Haecker, T. L., report of work on nitrogen determination by Mr. Anthony .. 181–183
Hamner, N. C., comments on humus studies 54
Hansen, A. W., and A. L. Winton, paper on "Effects of time and temperature of digestion on the amounts of acidity and nitrous nitrogen extracted from flour" 114–116
    L. C. Johnson, note on determination of tin in foods 117–118
Harrison, C. W., comments on determination of crude fiber in prepared mustard. 94
Hartwell, B. L., note on "Pot experiment showing gains in nitrogen when certain legumes were grown" 59–60
Haskins, H. D., and A. J. Patten, report on phosphoric acid 10–25
Headache mixtures, analysis, methods, etc 237–241
    report by W. O. Emery, associate referee 236–242
Henry, A. M., comments on vanilla extracts 134–135
Herles's solution, use as clarifying agent in sugar polarization 211
Hibbard, P. L., comments on phosphoric acid 17
    report on cobalti-nitrite method 36–38
Hill, H., comments on crude fiber determination 201
  H. H., comments and report on cobalti-nitrite method 33, 40
Hiltner, R. S., comments on ether extracts from cocoa products 160–161
Hiltner, R. S., note on tentative methods for determination of essential oil in alcohol solutions 195–196
    report as associate referee on flavoring extracts 127–158
Hilts, R. W., recalculation of Juckenack's egg-noodle tables 116–117
    report as associate referee on spices 89–95
Hoagland, Ralph, associate referee on meat and fish 213–217
"Homogenizer," use in adulterating dairy products 100

Page.
Horne, W. D., paper on "Temperature corrections in raw sugar polarizations". 207–210
Horne's method, sugar clarification, status........ 211
Humus, studies, report by G. S. Fraps........ 52–53

Insecticides, recommendations........ 71–72, 85–86
report by C. C. McDonnell, referee........ 68–72
Iron, determination in mineral waters........ 78
Itano, Arao, comments and report on cobalti-nitrite method........ 33, 39–40

Jaffa, M. E., report as associate referee on tea and coffee........ 163–167
Jams, analysis, method........ 168–169
Jellies, analysis, method........ 169
Jelly powders, analysis, method........ 169
Johnson, L. C., and A. W. Hansen, note on determination of tin in foods.... 117–118
Juckenack's egg-noodle tables, recalculation, by R. W. Hilts........ 116–117

Kebler, L. F., paper on "Standard for tincture of ginger"........ 244–248
report as referee on medicinal plants and drugs........ 234–236
verbal report as chairman chemical reagents testing committee. 87
Kellogg, James W., comments on crude fiber determination........ 201
report as referee on nitrogen........ 27–28
Kelly, W. P., comments on humus studies........ 54
Kennedy, M. B., comments on flavoring extracts........ 135, 138
Kerr, R. H., comments on detection of beef fat in lard........ 99
Ketchup, work, methods of examination........ 118–122
Ketchups, from sound and unsound tomatoes, comparison of analyses...... 120–122
work........ 88–89
Kimberly, C. H., paper on "Standard for tincture of ginger"........ 244–248
Kjeldahl-Gunning method for separation of nitrogenous bodies........ 184

Lard, beef fat content, detection work........ 96–100
Le Febvre, C. C., comments on headache mixtures........ 238
Lead arsenate, analysis........ 68–70
Leather analysis, plan of work, samples, etc........ 221–223
recommendations........ 231–233
results and comments........ 221–231
extraction, analysis of extract, etc........ 224–225
Leucosin, determination in flour........ 104
Liepsner, F. W., comments on vanilla extracts........ 134
Lime, determination in powdered ginger........ 247
Lime-sulphur solutions, sulphur determination........ 70–71
Lipman, J. G., and G. S. Fraps, report on soils........ 50–56
report on soil-estimation methods........ 50–52
Lipscomb, G. F., comments on cobalti-nitrite method........ 34
Liqueurs, benzaldehyde determination, note by F. G. Smith........ 192–195
Liquors, distilled, note by F. G. Smith........ 192–195
Lithium, determination in mineral waters........ 80–81
Loomis, H. M., comments on headache mixtures........ 238
Lott, C. I., comments on ether extract from cocoa products........ 160
Lowenstein, A., comments on nitrogen determination........ 180–181

MacNider, G. M., comments on fat determination........ 199
report as referee on feeds and feeding stuffs........ 197–202
Magnesium, determination in mineral waters........ 79
Manganese, determination in mineral waters........ 78–79

Page.

Manganese, determination method, studies by W. H. McIntyre, associate referee ... 66
Manure, potash content, determination ... 184–185
Marmalades, analysis, methods ... 168–169
Mathewson, W. E., report as associate referee on colors ... 122–124
McClelland, Byron, comments on ginger extracts ... 138
McCreary, P. L., comments on phosphoric acid ... 17
  report on cobalti-nitrite method ... 38–39
McGee, W J, report as associate referee on condiments other than spices ... 118–122
McIntyre, W. H., ferric and aluminic oxids, work as associate referee on ... 64
  manganese determination, work and studies as associate referee ... 66
McLean, H. C., comments on humus studies ... 55
Meat, adulteration, problems ... 214
  proteids, separation, report by C. R. Moulton, referee ... 176–184
  proteins, recommendations of committee C ... 188
  report of Ralph Hoagland, associate referee ... 213–217
Meats, putrefaction, incipient, problem ... 214
Medicinal plants, recommendations of referee adopted ... 212
  report by L. F. Kebler, referee ... 234–236
Melzer method for detection of benzaldehyde and phenol ... 195
Members present at convention, list ... 5–9
Milk, recommendations on ... 86
  separation of nitrogenous bodies, report by A. W. Bosworth, associate referee, and O. B. Winter ... 185–187
  solids, determination in cocoa products ... 162–163
Mineral waters, analysis methods, recommendations ... 77–82
Mitchell, A. S., report as referee on food adulteration ... 88–89
  L. C., comments on ether extract from cocoa products ... 160
Moisture determination in flour, methods ... 101–102, 107
  molasses ... 203–205, 206–207
  sugar ... 205–207
Molasses, moisture content, determination studies and results, 1910–11 ... 203–205, 206–207
  recommendation ... 207
  report by William E. Cross, referee ... 202–207
Morphin, quantity estimation in mixtures, methods, report by E. O. Easton. 242–244
  recommendations ... 244
Moulton, C. R., report as referee on the separation of nitrogenous bodies ... 176–184
Murray, A. G., paper on "Assay of nitroglycerin tablets" ... 248–250
Mustard, prepared, crude fiber content, determination methods ... 91–95

New York food and drug inspection laboratory, results ... 216–217
Nitric acid, determination in mineral waters ... 81–82
  method, tin determination ... 214, 215
Nitrites, determination in flour, methods, note ... 196
Nitrogen, amid, determination in flour ... 104
  determination methods, work, studies, etc ... 27–28
    reports of referees ... 177–184
  gain in growth with legumes, experiments, paper by B. L. Hartwell and F. R. Prember ... 59–60
  nitrous, determination in flour ... 113–114
  recommendations ... 83
  report by James W. Kellogg, referee ... 27–28
  total, determination in flour, note ... 104, 109

Page.
Nitrogenous bodies (meat proteins), recommendations of committee C........ 188
separation from milk and cheese, report by A. W. Bosworth, associate referee, and O. B. Winter.. 185–187
report by C. B. Moulton.................... 176–184
Nitroglycerin, determination methods, etc.................................. 248–250
tablets, assay methods, etc.................................. 248–250
paper by A. G. Murray.......................... 248–250
Nominations, committee, report.. 220
Nutmeg extracts, cooperative work.. 139–141

Officers, association, for 1911–12, directory................................. 251–253
election and appointment... 220
Oil, essential, determination in alcohol solutions by R. S. Hiltner.......... 195–196
Oils, analysis, unification of methods, report of committee................ 169–170
recommendations of committee C.................................. 191–192
report by H. S. Bailey, associate referee............................. 96–100
Opium, powdered, morphin estimation.. 242–244
Oxids, ferric and aluminic, recommendations................................ 66–67
separation in ash solution, methods, etc.......... 60–66

Paprika, ether extract, drying experiments...................................... 89–90
index of refraction................................. 95–96
extract, detection of foreign oils, work 90–91
studies.. 89–91
Paregoric, morphin estimation.. 242–244
Patten, A. J., and C. S. Robinson, article on "Neutral ammonium-citrate solution".. 25–27
H. D. Haskins, report on phosphoric acid................ 10–25
Paul, A. E., article on "Methods of analysis of fruit products"............ 168–170
report as associate referee on adulteration of dairy products.... 100–101
Pettit, J. H., comments on humus studies.. 55–56
Phosphates, efficiency comparisons, experiments at Massachusetts Experiment Station.. 18–25
slag, studies, review of work, recommendations, etc...... 10–18, 24–25
Phosphoric acid, determination in soft drinks................................ 241–242
recommendations..................................... 24–25, 83
report by H. D. Haskins, referee, and A. J. Patten, associate referee.. 10–25
Pimenton, ether extract, index of refraction.................................. 95–96
Pinckney, R. M., report on cobalti-nitrite method................................ 36
Pinkerton, Thos. C., comments and report on cobalti-nitrite method.......... 34–36
Plant constituents, inorganic, report by O. M. Shedd, referee.............. 60–67
Plants, medicinal, recommendations.. 235–236
report by L. F. Kebler, referee......................... 234–236
Polarization, sugar and molasses, effects of clarifying agents................ 206–207
details of work... 208–210
temperature corrections........................... 207–210
Poore, Homer D., comments on detection of beef fat in lard..................... 99
Porter, F. B., comments on cobalti-nitrite method.............................. 35
Potash, determination and value, notes, by P. F. Trowbridge.............. 184–185
recommendations.. 41–42, 84
report by E. L. Baker, referee... 28–42
studies and work, comments by analysts.................................. 33–50

Page.

- Potassium, cobalti-nitrite method, article by Leon T. Bowser ... 45–50
  - determination in mineral waters ... 80–81
  - estimation as cobalti-nitrite, article by W. A. Drushel ... 42–45
- Prember, F. R., note on "Pot experiment showing gains in nitrogen when certain legumes were grown" ... 59–60
- Preservatives, report by H. E. Barnard, associate referee ... 167
- President's address to association ... 172–176
- Printing bill, food standards committee, payment authorized ... 87
- Proulx, E. G., comments on phosphoric acid ... 16
- Putrefaction, incipient, in meats ... 214

- Rather, J. B., comments on humus studies ... 54
- Ray, B. J., comments on leather analysis ... 226–227
- Recommendations, coffee and tea analysis ... 166
  - examination of spices ... 95
  - extraction of fat from dairy products ... 101
  - feeding stuffs ... 201–202
  - ferric and aluminic oxids ... 66–67
  - humus studies and methods ... 56
  - insecticides ... 71–72
  - ketchups ... 122
  - leather analysis ... 231–233
  - medicinal plants and drugs ... 235–236
  - molasses ... 207
  - morphin estimation in mixtures ... 244
  - nitrogen determination ... 183–184
  - of referees, report of committee A ... 83–86
    - B ... 210–212
    - C ... 188–192
  - phosphoric acid determination ... 24–25
  - potash ... 41–42
  - separation of nitrogenous bodies in milk and cheese ... 187
  - soils ... 83–84
  - spices ... 188
  - sugar analysis ... 207
  - tannin ... 231–233
  - vinegar examination ... 126
  - water analysis ... 77–82
  - wheat-flour examination ... 113
  - work on flavoring extracts ... 143–145
- Referees, for 1911–12, directory ... 251
  - recommendations, report of committees ... 83–86, 188–192, 210–212
- Refractometer method for moisture determination in molasses ... 203–204
- Resolutions, committee, report ... 213
- Robinson, C. S., and A. J. Patten, article on "Neutral ammonium-citral solution" ... 25–27
  - comments on humus studies ... 55
- Rogers, J. S., report as referee on tannin ... 221–233
- Roop, J. H., comments on gravimetric method ... 34
- Rudnick, Paul, comments on cobalti-nitrite method ... 35
  - detection of beef fat in lard ... 99
  - work on nitrogen determination ... 178–179

Page.

Sausage, adulteration with starch.... 214
 starch determination.... 214
Schneider, B. B., comments on detection of beef fat in lard.... 99
Schreiber and Taber, comments on tin determination.... 217
 tin determination method, objection.... 214
Secretary of Agriculture, address to convention, mention.... 197
Seeker, A. F., comments on detection of beef fat in lard.... 99
Seil, H. A., comments on morphin estimation in mixtures.... 243
Seltzer, J. M., comments on leather analysis.... 227
Shedd, O. M., comments on cobalti-nitrite method.... 38
 report as referee on inorganic plant constituents.... 60–67
 on potash determination.... 35–36
Silica, determination in mineral waters.... 77–78
Sindall, H. E., comments on determination of crude fiber in prepared mustard. 94
Sirup, special, morphin estimation.... 242–244
Skinner, W. W., report as referee on water.... 72–82
Slag, basic, studies, review of work, recommendations, etc.... 10–18, 24–25
Smith, C. R., comments on determination of crude fiber in prepared mustard.. 94
 F. G., note on determination of benzaldehyde in liquers, distilled liquors, and cordials.... 192–195
Sodium, determination in mineral waters.... 80–81
Soft drinks, analysis.... 241–242
 medicated, report by H. C. Fuller, associate referee.... 241–242
Soil acidity, estimation, bacteriological methods, report by J. G. Lipman, referee.... 50–52
 humus, studies, report by G. S. Fraps.... 52–53
Soils, carbon dioxid determination, paper by Leon T. Bowser.... 56–59
 recommendations.... 83–84
 report by J. G. Lipman, referee, and G. S. Fraps, associate referee.... 50–56
Spices, recommendations of committee C.... 188
 report by R. W. Hilts, associate referee.... 89–95
Starch, determination in powdered ginger.... 247
 sausage.... 214
 use in sausage, as adulterant, problem.... 214
Stevenson, A. E., comments on morphin estimation in mixtures.... 243
Stewart, G. R., and A. R. Mehrtens, comments on analysis of tea and coffee. 165–166
 determination of caffein in coffee.... 165–166
Stillwell and Gladding, comments on ferric oxid.... 64
Street, J. P., comments on headache mixtures.... 238
 report of committee A on recommendations of referees.... 83–86
 verbal report as chairman appropriations committee.... 87
 applied chemistry congress committee. 87
Strontium, determination in mineral waters.... 79
Sugar analysis methods, instructions, and results.... 202–207
 recommendations.... 207
 moisture content, determination studies, comparison of methods, etc.. 205–207
 polarization, effects of clarifying agents.... 206–207
 temperature corrections.... 207–210
 raw, moisture content, analysis.... 206
 recommendations of referees disposed of.... 211
 report by William E. Cross, referee.... 202–207
 trade laboratory, polarizations.... 208, 210

Page.
Sugars, determination in flour, Bryan, Given, and Straughn method 103
    raw, polarization comparisons, tables 209, 210
Sulphur, determination in lime-sulphur solutions 70–71
Sulphuric acid, determination in mineral waters 80
        method, in determination 215

Tannin, recommendation of referee adopted 212
    recommendations 231–233
    report by J. S. Rogers, referee 221–233
Tea, analysis, methods 164
    cooperative work 164
    report by M. E. Jaffa as associate referee 163–167
Temperature corrections in raw sugar polarizations, paper by W. D. Horne 207–210
Thein, determination in tea 164
Tin determination, canned goods, comments 217
            recommendation 217
        in foods, method, by A. W. Hansen and L. C. Johnson 117–118
    limit in canned goods 214
    results of determinations 215–217
Tolman, L. M., report on standardization of alcohol tables 169
            unification of methods of analysis of fats and oils 169–170
Tonka bean, composition, note 146–147
Trescot, T. C., work on nitrogen determination 181
Treuthardt, E. L. P., comments on flavoring extracts 138, 140
Trowbridge, P. F., comments on cobalti-nitrite method 35
        notes on determination and value of potash 184–185

Vanatta, E. E., work on nitrogen determination 179–180
Vanilla beans, varieties 146–147
    extract, analysis, methods 147–149
        cooperative work, methods, etc 128–136
        variation in composition 146
    extracts, analyses 150–158
        authentic, chemical composition, analytical methods, and limits of constants 146–158
        comments of analysts 133–135
        manufacture, processes 147
        preparation 149–150, 156–157
    La Guayra, comparison with vanilla bean 146
    pompona, comparison with vanilla bean 146
    samples for examination, description 129
Vanillons, comparison with vanilla bean, use, etc 146
Van Slyke, L. L., report as chairman of committee on amendments to constitution and by-laws 86–87
Vinegar analysis, methods 126–127
    recommendations of committee C 188–189
    report by W. A. Bender, associate referee 125–127
Visitors present at convention, list 5–9
Vollertsen, J. J., work on nitrogen determination 181

Walker, G. W., comments on humus studies 54–55
Water extracts of flour, acidity determination 102–108
    in foods, recommendations of committee C 188
        report by Herman C. Lythgoe, associate referee 167–168

Page.
Water recommendations 84
  report by W. W. Skinner, referee 72–82
Waters, mineral, analysis methods, recommendations 77–82
Weatherhead, D. L., work on index of refraction of ether extract of paprika and pimenton 95–96
White, H. L., report as associate referee on cereal products 101–113
Whitehouse, W. L., comments on phosphoric acid 17
Wiley, H. W., report as chairman committee on cooperation with other agricultural organizations 170–171
Willard, J. T., report on nitrogen determination by J. W. Calvin 177
Winter, O. B., and A. W. Bosworth, report on separation of nitrogenous bodies in cheese and milk 185–187
Wintergreen extracts, cooperative work 141–142
Winton, A. L., and A. W. Hansen, paper on "Effects of time and temperature of digestion on the amounts of acidity and nitrous nitrogen extracted from flour" 114–116
    E. H. Berry, article on "Chemical composition of authentic vanilla extracts, analytical methods, and limits of constants" 146–158
  comments on vanilla extracts 134
  paper on "Nitrous nitrogen determination in flour" 113–114
  report as chairman of committee C 188–192
Withers, W. A., report as acting chairman of committee on nominations 220
Woll, F. W., address as president 172–176
Woodruff, F. O., comments on determination of caffein in coffee 165

Yoder, P. A., comments on ferric oxid 64

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